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RESPONSE OF PHYMATOTRICHUM OMNIVORUM TO CERTAIN TRACE ELEMENTS[1]

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INTRODUCTION

The cotton root rot disease, caused by *Phymatotrichum omnivorum* (Shear) Duggar, is one of the most serious plant diseases occurring in the southwestern part of the United States. Investigations by numerous workers over the last half century suggest that this disease-producing fungus is not amenable to the methods of control commonly and successfully applied to other plant pathogens. It is evident that a more exact knowledge of the relation of the organism to its environment, both biological and chemical, must be obtained before a satisfactory explanation can be had of the many obscure problems dealing with the organism and its action upon the host plant. The studies here reported constitute one phase of a general investigation of the nutritional requirements of *P. omnivorum* and deal primarily with the response of the organism to a number of the trace elements, including copper, iron, manganese, zinc, aluminum, boron, cadmium, cobalt, fluorine, mercury, iodine, lithium, molybdenum, nickel, and silicon. While the possibility that certain of these elements may be highly toxic to the root rot organism is a matter of practical interest, it is equally important to know the contribution of each of the elements to an optimum nutrient solution. Such information may be used in connection with the general problem of nutrition and other physiological investigations.

In the extensive literature dealing with cotton root rot and its causal organism, little is to be found regarding the effect of these elements on the fungus. Rogers (*5*)[3] in laboratory experiments found that copper had a stimulating effect at low concentrations but was toxic at higher concentrations, zinc stimulated growth at all concentrations up to and including 200 p. p. m., while aluminum and mercury were toxic at concentrations above 50 p. p. m. Taubenhaus, Ezekiel, and Fudge (*10*) reported that the addition of manganese,

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[3] Italic numbers in parentheses refer to Literature Cited, p. 159.

sulfur, iodine, iron, nickel, copper, and boron to soils was without effect on the incidence or severity of root rot.

The investigations of Steinberg (*7*, *8*, *9*) with *Aspergillus niger* Van Tiegh. have furnished considerable information on the influence of many of the elements upon that fungus. With the refinement by Steinberg of the technique for the removal of traces of impurities from the nutrient solution it has become possible to study more precisely the effect of the addition of the various elements upon fungus growth. Foster (*4*) has recently reviewed the literature dealing with the effects of heavy metals or trace elements on various fungi.

MATERIALS AND METHODS

Preliminary experiments demonstrated that the nutrient solution designated as No. 70 by Ezekiel, Taubenhaus, and Fudge (*2*) was very favorable for growth of the root rot fungus, and with but slight modification it has been used in these studies, as follows:

Constituent:	*Grams per liter*
NH_4NO_3	1.00
K_2HPO_4	1.35
$MgSO_4.7H_2O$	.75
KCl	.15
$FeCl_3.6H_2O$	.00223
Glucose	40.0
Redistilled water to make to 1,000 cc.	

The following salts were used in studying the various trace elements: Copper sulfate ($CuSO_4$), iron sulfate ($FeSO_4.7H_2O$), manganese sulfate ($MnSO_4.4H_2O$), zinc sulfate ($ZnSO_4.7H_2O$), aluminum sulfate ($Al_2(SO_4)_3$), potassium borate ($K_2B_4O_7.5H_2O$), cobalt sulfate ($CoSO_4.7H_2O$), potassium fluoride ($KF.2H_2O$), mercuric chloride ($HgCl_2$), potassium iodide (KI), lithium sulfate ($Li_2SO_4.H_2O$), ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$), nickel sulfate ($NiSO_4.6H_2O$), sodium silicate ($Na_2SiO_3.9H_2O$), and cadmium sulfate ($CdSO_4$).

In order to evaluate the effect of the addition of each of these elements to the nutrient solution, it was necessary to remove, so far as possible, all traces of these elements that might have been introduced into the solution as impurities in the c. p. grade of chemicals used in the preparation of the solution. For such purification, calcium carbonate was added to the solution at the rate of 15 gm. per liter, followed by autoclaving and filtering through fritted glass crucibles, after the method of Steinberg (*7*). To the filtered aliquots of the purified solution the elements were added, and the solution was then divided into 50-cc. portions in 250-cc. Erlenmeyer flasks and autoclaved at 15 pounds pressure for 15 minutes. The treatments were ordinarily set up in series of five flasks.

The flasks of nutrient solution were inoculated with 5-mm. disks of mycelium and adhering agar from potato-dextrose agar plate cultures of the organism. The dry weight of the inoculum piece was usually less than 5 mg., varying with the depth of the agar in the transfer plate. In the preliminary phases of the study a pure culture of *Phymatotrichum omnivorum* designated as isolate 41 was used, but in the later and more extensive experiments a newly isolated culture designated as isolate 28 was the source of the inoculum. Both cultures were of proved pathogenicity upon cotton seedlings.

The incubation of all cultures was at 28° C. in incubators or in a constant-temperature room, the period of incubation being 28 days in the majority of the experiments. The acidity of the solution in the culture flasks was determined with a Beckmann pH meter prior to inoculation and in many of the experiments at time of harvest. The fungus mats were harvested by filtering through Alundum crucibles and drying to constant weight at 80° C., either in crucibles or in weighing bottles. The dry weight of the fungus mat was the criterion of response to the addition of the various elements.

RESULTS

EFFECT OF ADDING ELEMENTS SINGLY

The effect of adding certain elements (copper, iron, manganese, and zinc) to the purified nutrient solution was studied in a series of experiments, the range of concentrations being from 0.5 to 160 p. p. m. In the upper portion of table 1 are presented the data from one typical experiment in the lower range of concentrations. With the addition of iron, manganese, or zinc, increases in mat weight were obtained over that of the control solution to which no element was added. The addition of copper at the rates of from 0.5 to 10 p. p. m. had no appreciable effect upon the mat weight; at a concentration of 20 p. p. m. growth was almost completely inhibited.

Other experiments were conducted in which the addition of each of these elements to the purified solution was studied at higher rates. The addition of copper at 40, 80, or 160 p. p. m. completely inhibited growth of the organism. Iron or manganese at these rates resulted in decreases in the amount of mat weight as the concentration increased, eventually yielding less than the control series. With the addition of zinc at similar rates, decreases resulted at the higher concentrations, but the weights were superior to those of the control flasks. It was noted that at concentrations beyond 20 p. p. m. a precipitate was formed in the nutrient-solution flasks following autoclaving, and it is probable that with this slightly acid nutrient solution only a part of the addition was held in solution. Therefore, with the heavy rates of addition the amount of the element remaining in solution is not known.

The determinations of pH values of the solutions prior to inoculation showed that the reaction of those solutions receiving zinc or manganese deviated only slightly from that of the solution (table 1) to which no metals were added. The addition of iron or copper resulted in a slightly more acid condition. The final pH values of each flask at time of harvest showed a general trend toward increased acidity. In this experiment as well as in others, no relationship was found between the initial pH value of the solution and the mat weight. The possibility of such a relation in solutions having a greater initial range of pH values is not excluded, but with the nutrient solution used and an initial pH value of from 5.5 to 6.5 following the addition of the several elements, the effect of slight variation in the initial pH value upon subsequent growth has been greatly overshadowed by the effect of the element or combination of elements employed in the solution. It has been reported by other workers with this organism and with practically the same nutrient solution (*2*) that *Phymatotrichum omnivorum* will grow in solutions of widely varying hydrogen-ion

concentration, and that, although the optimum pH value for growth appeared to be on the alkaline side of neutrality, growth was almost equally good over a range of approximately pH 4.0 to 9.0. In the present study no attempt was made to adjust the pH value of the nutrient solution.

TABLE 1.—*Effect of adding certain elements, singly and at several rates, to a purified solution on the growth of Phymatotrichum omnivorum at 28° C. for 28 days*

Element added	Rate of addition	Original pH	Final pH of replicate—				Mat weight				
							Replicate—				Mean
			a	b	c	d	a	b	c	d	
	P.p.m.						*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Copper	0.5	5.92	5.70	5.83	5.60	5.79	166.7	139.8	159.7	169.6	159.0
	1.0	5.88	5.53	5.57	5.72	6.02	195.7	196.4	168.0	126.7	171.7
	2.0	5.83	5.65	5.71	5.56	5.96	167.3	197.2	90.5	113.1	142.0
	5.0	5.83	5.62	5.55	5.55	5.58	193.2	185.2	176.1	120.2	168.7
	10.0	5.80	5.61	5.37	5.40	5.45	179.0	149.4	158.3	141.9	157.2
	20.0	5.65	4.93	4.45	4.42	4.42	36.2	41.1	40.2	45.0	40.6
Iron	.5	6.04	5.60	5.79	5.84	5.67	274.0	229.7	272.8	241.7	254.6
	1.0	6.01	5.93	5.84	5.80	5.72	260.3	218.0	220.0	243.0	235.3
	2.0	5.99	5.34	5.62	5.66	5.57	304.9	229.9	327.4	202.7	266.2
	5.0	5.94	5.49	5.40	5.56	5.50	224.0	265.0	235.0	246.9	242.7
	10.0	5.93	5.40	5.34	5.19	5.23	245.4	220.7	150.6	189.6	201.6
	20.0	5.90	5.51	5.55	5.06	4.92	186.8	186.5	196.5	177.2	186.8
Manganese	.5	6.10	5.28	5.40	5.61	5.48	265.6	255.1	280.4	271.6	268.2
	1.0	6.13	5.30	5.47	5.50	5.54	259.0	246.8	247.3	240.4	248.4
	2.0	6.10	5.58	5.51	5.50	5.69	194.5	186.6	214.2	177.8	193.3
	5.0	6.17	5.52	5.50	5.39	5.51	166.4	232.1	170.9	120.6	172.5
	10.0	6.20	5.64	5.36	5.50	5.34	172.3	198.8	252.0	174.7	199.5
	20.0	6.13	5.20	5.27	5.44	5.33	252.4	231.9	230.4	251.6	241.6
Zinc	.5	6.14	5.60	6.21	6.48	4.26	345.1	315.1	371.3	406.9	359.6
	1.0	6.20	4.49	5.01	4.92	4.65	403.7	365.1	305.1	355.4	357.3
	2.0	6.20	5.10	5.50	5.90	.....	373.9	311.1	313.7	347.5	336.6
	5.0	6.18	5.40	5.56	5.43	6.18	332.7	302.4	300.9	298.7	308.7
	10.0	6.12	5.76	5.80	5.73	5.32	360.7	313.9	303.5	298.7	319.2
	20.0	6.05	6.10	5.93	6.06	6.00	258.3	357.1	306.1	283.7	301.3
None	.....	6.09	5.41	5.41	5.40	5.38	167.6	134.3	179.7	169.8	162.8

Element added	Rate of addition	Mat weight					
		Replicate—					Mean
		a	b	c	d	e	
	P.p.m.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Aluminum	1	208.0	140.0	173.9	198.5	158.8	175.8
	10	130.7	85.7	82.8	120.5	106.5	105.2
	20	124.1	106.3	73.5	133.8	81.3	103.8
	40	88.3	72.7	63.6	80.2	60.0	73.1
Cadmium	1	260.7	213.6	134.0	196.4	156.9	192.3
	10	96.3	106.4	99.4	169.6	124.7	119.3
	20	53.7	69.1	77.0	56.1	66.6	64.5
	40	38.1	52.0	45.9	41.0	49.3	45.3
Cobalt	1	201.5	175.8	178.6	174.4	177.2	181.5
	10	25.9	25.3	24.5	22.1	(1)	24.5
	20	15.2	7.5	15.6	14.0	10.8	12.6
	40	11.7	11.1	10.9	10.7	8.4	10.6
Nickel	1	117.2	152.4	160.3	166.1	127.9	144.8
	10	37.1	33.3	89.8	115.9	19.5	59.1
	20	7.4	6.8	9.1	7.6	6.0	7.4
	40	6.4	6.1	6.6	6.6	4.0	5.9
Copper	1	163.5	152.2	168.5	167.6	144.6	159.3
	10	198.3	99.1	131.6	112.9	136.0	135.6
	20	27.0	9.9	13.2	12.5	10.1	14.5
	40	4.0	2.1	5.2	4.4	(1)	3.9
Zinc	1	479.2	504.4	522.3	586.3	468.7	512.2
	10	502.8	563.6	555.8	531.9	550.0	540.8
	20	522.7	532.1	577.2	569.9	555.9	551.6
	40	492.3	541.9	498.3	530.8	526.0	517.9
None	.....	191.2	120.5	139.3	157.1	181.5	157.9

[1] Contaminated and discarded.

The studies dealing with the effects of copper, iron, manganese, and zinc upon the growth of *Phymatotrichum omnivorum* were extended to include the effects of aluminum, boron, cobalt, cadmium, fluorine, iodine, lithium, mercury, molybdenum, nickel, and silicon. To the standard nutrient solution, purified by the calcium carbonate treatment, these elements were added to give concentrations of 1, 4, 10, and 20 p. p. m. The addition of these elements singly at these rates failed to give significant increases in mat weight. Definite inhibition of growth to various degrees occurred with the addition of cobalt at 4, 10, and 20 p. p. m., and with nickel at 10 and 20 p. p. m.; aluminum and cadmium showed only a mild inhibition at 4, 10, and 20 p. p. m. The remaining elements appeared to exert slight, if any, influence upon growth of the organism.

To study further and in more detail the action of certain of the elements, an experiment was conducted with the purified solution in which the addition of cobalt, nickel, aluminum, or cadmium was compared with the addition of copper or zinc. The rates of addition were at 1, 10, 20, and 40 p. p. m. The results are summarized in the lower portion of table 1. As usual, copper almost completely inhibited growth at 20 p. p. m., while zinc at all concentrations caused a great increase in mat weight. Both cobalt and nickel were highly toxic to the organism at the two higher concentrations, and a marked depression of growth was displayed at the concentration of 10 p. p. m. As in earlier experiments aluminum and cadmium caused a mild depression of growth at the higher range of concentrations. No significant increases in mat weight occurred, except with the addition of zinc.

EFFECT OF ADDING ELEMENTS IN COMBINATION

The effect of adding copper, iron, manganese, and zinc was studied in experiments where all were added to the purified solution or to the unpurified solution, the majority of the experiments being conducted upon the purified solution. The rates of addition ranged from less than 1 p. p. m. to 40 p. p. m. of each element. The lower rates of addition were based upon certain concentrations used by Steinberg (7) in studies with *Aspergillus niger;* that is, copper at 0.12, iron at 0.40, manganese at 0.06, and zinc at 0.28 p. p. m. While increases in mat weight over those obtained in the controls were had by the addition of these elements to the purified solution at these rates, or even at lesser concentrations, it was found that by increasing the rate of addition better growth resulted, as indicated in table 2. Eventually all of these elements were studied in combination at the same rates of addition; that is, at 2, 5, 10, or 20 p. p. m. of each. It was demonstrated that the fungus responded almost equally well in the purified solution to additions of the elements over a range of from less than 1 p. p. m. to 10 p. p. m. The results indicate that, for the range of concentrations used in these experiments, *Phymatotrichum omnivorum* does not have a sharply defined optimum requirement as concerns these elements. With the elements added at the rate of 20 or more p. p. m. growth was depressed directly as the concentration of the elements was increased. It is apparent that when all four elements were present in the solution at 20 p. p. m. of each, the toxic effect of copper (table 1) was largely overcome by the other elements, but at concentrations of 40 p. p. m., almost complete failure of growth occurred.

TABLE 2.—*Summary of experiments on the effect of adding copper, iron, manganese and zinc, at various concentrations, upon the growth of Phymatotrichum omnivorum in purified solution, incubated at 28° C. for 28 days*

Experiments (number)	Rate of addition of—				Flasks	Mean weight of mats	Control (no element added)	
	Cu	Fe	Mn	Zn			Flasks	Mean weight of mats
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*Number*	*Mg.*	*Number*	*Mg.*
5	0.12	0.40	0.06	0.28	27	603.0	29	151.1
4	.48	1.60	.24	1.12	24	647.2	23	155.9
9	2.0	2.0	2.0	2.0	43	709.7	41	164.6
8	5.0	5.0	5.0	5.0	37	716.3	36	171.2
4	10.0	10.0	10.0	10.0	18	661.5	18	173.7
2	20.0	20.0	20.0	20.0	7	456.9	8	172.3
2	40.0	40.0	40.0	40.0	8	15.5	8	172.3

Several experiments were conducted in which the four elements in combination were added to the nutrient solution which had not been purified by the calcium carbonate treatment, the range of addition being from less than 1 p. p. m. to 10 p. p. m. of each element. Although increases in mat weight over those obtained in the control flasks resulted from the addition of the elements, the increases were not so great as had occurred with a similar addition to the purified solution. For example, the addition of elements to the unpurified solution yielded mats of 500 mg. or less, whereas a similar addition to the purified solution gave mats weighing 600 to 700 mg. As was to be expected, the controls with the unpurified solution consistently yielded heavier mats than did the controls with the purified solution, indicating the efficiency of the calcium carbonate treatment in the removal of traces of these elements.

It should be observed that in none of the experiments with the purified solution were the mean weights of the controls as low as those reported by Steinberg in his studies with *Aspergillus*. Inasmuch as *Phymatotrichum omnivorum* does not produce a germinable conidial spore, the inoculum used in the present studies consisted of disks of mycelium and adhering agar from agar plate cultures of the organism. Such inoculum, usually 3 to 5 mg. in dry weight, undoubtedly carried traces of the essential elements in the mycelium or in the agar, thus accounting in part for the considerable growth that occurred in the control flasks.

FACTORIAL-DESIGN EXPERIMENTS

METHOD OF STUDYING INTERACTIONS

In the discussion of the preceding experiments, it was pointed out that, where copper, iron, manganese, and zinc had been added singly to the purified solution, zinc roughly doubled, or more than doubled, the mat weight over that of the control, whereas iron and manganese gave smaller increases, the weights being intermediate between those of zinc and the controls (table 1). Copper gave no consistent increases, although in certain cases it appeared to be slightly beneficial. However, when these elements were used in combination, that is, when all four were present at various levels ranging from less than 1 p. p. m. to 10 p. p. m., the mat weights obtained were approximately four times as great as the controls, indicating the possibility of important interactions. It was therefore deemed advisable to conduct a series of experiments designed to permit a study of these interactions.

Factorial experiments, initiated by Fisher (*3*), developed by Yates (*11*), and presented in systematic form by Brandt (*1*), are particularly suitable for studies of the type mentioned in the preceding paragraph. The essential principles of this type of experiment require that the factors being investigated, in this case copper, iron, manganese, and zinc, be set up in all possible combinations at the given levels of the experiment. In these experiments with 4 elements, 16 combinations or treatments are possible, in which one or more of the elements are present or absent, at a given rate. In the analysis of such an experiment, all combinations of factors could be restratified for studying all combinations and interactions. Experiments of this type were conducted, the elements being added at the rate of 2, 5, or 10 p. p. m. Throughout the course of these studies precautions were taken to make the several experiments as nearly comparable as possible. The nutrient solution was prepared from stock solutions of the various salts; the purification technique was standard in all; the elements were added at the proper amounts to give the desired concentration; and the inoculum and incubation temperatures were as uniform as possible from experiment to experiment. The various treatments, or combinations of elements, were prepared on the basis of five replicates for a given combination, but losses through contamination or other causes sometimes reduced the number to four by time of harvest. The replicates of a given combination were assigned numbers at random, and were randomized in their location in the incubation chamber. Hence, in analyzing the results of an experiment, no degrees of freedom were lost for stratification of replicates.

Effect of Adding Four Elements Singly and in Combination

Of a number of such experiments in factorial design, three have been selected as typical of the results obtained at the rates of 2, 5, and 10 p. p. m. respectively. Experiment 91, at 5 p. p. m., was based upon four replicates of each treatment, while experiments 108 and 127, at 10 and 2 p. p. m. respectively, were based upon five replicates of each treatment or combination. The incubation period of experiment 127 was 21 days, while that of the others was, as usual, 28 days.

Separate analyses were made of each experiment. The three experiments were also brought together into a combined analysis, and this is presented along with the separate analyses in tables 3 and 4. The effects of rates of addition of copper, iron, manganese, and zinc, and the interactions involving rates, could be determined only from such a combined analysis. In discussing the results of the three experiments and their separate and combined analyses, the several sources of variance will be dealt with in the order of their appearance in tables 3 and 4.

Rates.—It will be noted from the mean square, as well as from the mean mat weights, that the difference between the average mat weight at 2 p. p. m. and at 10 p. p. m. (R_1) is enormous. As mentioned above the harvest for the experiment at 2 p. p. m. (experiment 127) was at, 21 days instead of 28 days as in the experiments at 5 and 10 p. p. m. This would account for the consistently lower mat weights obtained at the 2-p. p. m. rate of addition. On the other hand, R_2, the mean value for rates at 2 and 10 p. p. m., compared with that at 5 p. p. m. does not give significant differences. It should also be borne in mind that since these were separate experiments, rates may be confounded

with such factors as failure to maintain absolutely identical temperatures, differences in vigor of the inoculum, or other factors that are beyond the operator's control. The mean weight at 5 p. p. m. is lower than the value obtained at 10 p. p. m., despite the fact that copper at 10 p. p. m. was somewhat detrimental. In previous experiments it was shown that optimum growth (table 2) occurred at 2 and 5 p. p. m., with little distinction between these rates, and that growth at 10 p. p. m. was only slightly less than that at 2 and 5 p. p. m.

TABLE 3.—*Mean values for the effect of copper, iron, manganese, and zinc on growth of Phymatotrichum omnivorum (experiments 127, 91, and 108) at rates of 2, 5, and 10 p. p. m. respectively, in purified solutions*

Contributor	Mean mat weight at [1]—			
	2 p. p. m.	5 p. p. m.	10 p. p. m.	Combined
	Mg.	*Mg.*	*Mg.*	*Mg.*
Cu×Fe×Mn×Zn (× rates):				
Cu absent:				
Fe absent:				
Mn absent:				
Zn absent	88	157	134	124
Zn present	126	321	422	288
Mn present:				
Zn absent	116	185	282	195
Zn present	153	381	489	338
Fe present:				
Mn absent:				
Zn absent	116	197	416	246
Zn present	516	518	589	543
Mn present:				
Zn absent	155	227	247	208
Zn present	522	755	788	684
Cu present:				
Fe absent:				
Mn absent:				
Zn absent	97	212	184	161
Zn present	154	339	483	324
Mn present:				
Zn absent	108	242	240	193
Zn present	204	453	528	391
Fe present:				
Mn absent:				
Zn absent	129	243	196	185
Zn present	560	552	699	607
Mn present:				
Zn absent	140	261	191	193
Zn present	558	645	758	655
Rates	234	355	415	.....
Cu×Fe×Mn (× rates):				
Cu absent:				
Fe absent:				
Mn absent	107	239	278	206
Mn present	134	283	386	266
Fe present:				
Mn absent	316	358	503	395
Mn present	338	491	518	446
Cu present:				
Fe absent:				
Mn absent	126	275	334	243
Mn present	156	347	384	292
Fe present:				
Mn absent	344	398	447	396
Mn present	349	453	475	424
Cu×Fe×Zn (× rates):				
Cu absent:				
Fe absent:				
Zn absent	102	171	208	159
Zn present	139	351	456	313
Fe present:				
Zn absent	135	212	332	227
Zn present	519	637	689	613
Cu present:				
Fe absent:				
Zn absent	103	227	212	177
Zn present	179	396	506	358
Fe present:				
Zn absent	134	252	194	189
Zn present	559	599	729	631

[1] For convenience, means in this table are given as nearest whole milligram, although tenths of a milligram were recorded and used in the analysis of results.

TABLE 3.—*Mean values for the effect of copper, iron, manganese, and zinc on growth of Phymatotrichum omnivorum (experiments 127, 91, and 108) at rates of 2, 5, and 10 p. p. m. respectively, in purified solutions*—Continued

Contributor	Mean mat weight at—			
	2 p. p. m.	5 p. p. m.	10 p. p. m.	Combined
	Mg.	*Mg.*	*Mg.*	*Mg.*
Cu×Mn×Zn (× rates):				
Cu absent:				
Mn absent:				
Zn absent	102	177	275	185
Zn present	321	420	506	415
Mn present:				
Zn absent	135	206	265	202
Zn present	337	568	639	511
Cu present:				
Mn absent:				
Zn absent	113	227	190	173
Zn present	357	446	591	466
Mn present:				
Zn absent	124	251	216	193
Zn present	381	549	643	523
Fe×Mn×Zn (× rates):				
Fe absent:				
Mn absent:				
Zn absent	93	184	159	142
Zn present	140	330	453	306
Mn present:				
Zn absent	112	213	261	194
Zn present	178	417	509	364
Fe present:				
Mn absent:				
Zn absent	122	220	306	216
Zn present	538	535	644	575
Mn present:				
Zn absent	147	244	219	201
Zn present	540	700	773	669
Cu×Fe (× rates):				
Cu absent:				
Fe absent	121	261	332	236
Fe present	327	424	510	420
Cu present:				
Fe absent	141	311	359	267
Fe present	347	425	461	410
Cu×Mn (× rates):				
Cu absent:				
Mn absent	211	299	390	300
Mn present	236	387	452	356
Cu present:				
Mn absent	235	336	390	319
Mn present	253	400	429	358
Cu×Zn (× rates):				
Cu absent:				
Zn absent	119	191	270	193
Zn present	329	494	572	463
Cu present:				
Zn absent	119	239	203	183
Zn present	369	497	617	494
Fe×Mn (× rates):				
Fe absent:				
Mn absent	116	257	306	224
Mn present	145	315	385	279
Fe present:				
Mn absent	330	378	475	395
Mn present	344	472	496	435
Fe×Zn (× rates):				
Fe absent:				
Zn absent	102	199	210	168
Zn present	159	373	481	335
Fe present:				
Zn absent	135	232	263	208
Zn present	539	618	709	622
Mn×Zn (× rates):				
Mn absent:				
Zn absent	107	202	233	179
Zn present	339	433	548	441
Mn present:				
Zn absent	130	229	240	197
Zn present	359	558	641	517
Cu (× rates):				
Cu absent	224	343	421	328
Cu present	244	368	410	339
Fe (× rates):				
Fe absent	131	286	345	252
Fe present	337	425	486	415

TABLE 3.—*Mean values for the effect of copper, iron, manganese, and zinc on growth of Phymatotrichum omnivorum (experiments 127, 91, and 108) at rates of 2, 5, and 10 p. p. m. respectively, in purified solutions*—Continued

Contributor	Mean mat weight at—			
	2 p. p. m.	5 p. p. m.	10 p. p. m.	Combined
	Mg.	*Mg.*	*Mg.*	*Mg.*
Mn (× rates):				
Mn absent	223	317	390	310
Mn present	244	394	441	357
Zn (× rates):				
Zn absent	119	215	236	188
Zn present	349	496	595	479

TABLE 4.—*Analysis of variance for effects of copper, iron, manganese, and zinc on growth of Phymatotrichum omnivorum in experiments 127, 91, and 108, at rates of 2, 5, and 10 p. p. m. respectively*

Variance	2 p. p. m.		5 p. p. m.		10 p. p. m.		Combined analysis	
	D. F.	Mean square [1]	D. F.	Mean square [1]	D. F.	Mean square [1]	D. F.	Mean square [1]
Rates: [2]								
R_1							1	*1, 319, 397
R_2							1	*43, 390
Cu	1	*7, 950	1	10, 499	1	2, 503	1	6, 108
R_1×Cu							1	9, 688
R_2×Cu							1	5, 156
Fe	1	*849, 153	1	*307, 706	1	*394, 735	1	*1, 494, 930
R_1×Fe							1	*42, 988
R_2×Fe							1	*13, 675
Mn	1	*9, 008	1	*92, 576	1	*50, 305	1	*124, 887
R_1×Mn							1	8, 369
R_2×Mn							1	*18, 633
Zn	1	*1, 064, 334	1	*1, 256, 053	1	*2, 567, 399	1	*4, 722, 630
R_1×Zn							1	*162, 818
R_2×Zn							1	2, 338
Cu×Fe	1	5	1	9, 754	1	*28, 861	1	*24, 230
R_1×Cu×Fe							1	*14, 051
R_2×Cu×Fe							1	339
Cu×Mn	1	264	1	2, 349	1	2, 573	1	4, 346
R_1×Cu×Mn							1	594
R_2×Cu×Mn							1	245
Cu×Zn	1	*7, 962	1	7, 857	1	*62, 658	1	*24, 192
R_1×Cu×Zn							1	12, 974
R_2×Cu×Zn							1	*41, 310
Fe×Mn	1	1, 130	1	5, 262	1	16, 574	1	3, 394
R_1×Fe×Mn							1	4, 524
R_2×Fe×Mn							1	*15, 048
Fe×Zn	1	*603, 208	1	*178, 686	1	*153, 186	1	*853, 768
R_1×Fe×Zn							1	*74, 218
R_2×Fe×Zn							1	7, 094
Mn×Zn	1	26	1	*39, 467	1	*36, 146	1	*46, 983
R_1×Mn×Zn							1	*19, 058
R_2×Mn×Zn							1	9, 599
Cu×Fe×Mn	1	527	1	11, 265	1	6, 151	1	556
R_1×Cu×Fe×Mn							1	5, 139
R_2×Cu×Fe×Mn							1	12, 248
Cu×Fe×Zn	1	7	1	4, 451	1	21, 783	1	2, 930
R_1×Cu×Fe×Zn							1	10, 504
R_2×Cu×Fe×Zn							1	12, 807
Cu×Mn×Zn	1	1, 180	1	1, 617	1	17, 173	1	6, 285
R_1×Cu×Mn×Zn							1	*13, 679
R_2×Cu×Mn×Zn							1	7
Fe×Mn×Zn	1	2, 148	1	6, 854	1	*86, 284	1	*36, 903
R_1×Fe×Mn×Zn							1	*57, 828
R_2×Fe×Mn×Zn							1	554
Cu×Fe×Mn×Zn	1	123	1	8, 600	1	*43, 866	1	*32, 890
R_1×Cu×Fe×Mn×Zn							1	*19, 674
R_2×Cu×Fe×Mn×Zn							1	25
Error	64	804	48	1, 897	64	3, 202	176	1, 974
Total	79	32, 893	63	32, 286	79	46, 774	223	43, 455
Mean square required for significance:								
Odds 99 : 1	------	5, 696	------	13, 713	------	22, 669	------	13, 344
Odds 19 : 1	------	3, 218	------	7, 701	------	12, 808	------	7, 679

[1] Asterisk (*) indicates that contributor is highly significant (odds 99 : 1). Calculations for determining significance were based on *F* values and *t* values taken from Snedecor's table (*6*).

[2] R_1 represents comparison of rates at 2 p. p. m. and 10 p. p. m.; R_2 represents mean value for rates at 2 and 10 p. p. m. as compared with 5 p. p. m.

Copper.—The presence of copper gave no highly significant increase over the values obtained in its absence except at 2 p. p. m., where the *F* value slightly exceeded the requirement for high significance.

Iron.—The presence of iron more than doubled the mat weight secured in the cultures from which iron was absent at 2 p. p. m., and was roughly one-half greater at both 5 and 10 p. p. m. The difference, of course, is highly significant at all rates.

Manganese.—The presence of manganese also gave a significant increase over the results obtained in its absence at all rates. The percentage of increase however, was relatively much smaller than that for iron.

Zinc.—At all rates the presence of zinc resulted in mat weights that exceeded by at least 100 percent those obtained in the absence of zinc.

Interactions.—It is exceedingly difficult to visualize interactions. Hence it seems desirable to supplement the tabular data with the scheme shown in figure 1 for presenting graphically the results obtained for experiment 108 at 10 p. p. m., in which significant interactions were obtained more frequently than at 5 and 2 p. p. m.[4]

Interaction of Cu×Fe.—Interaction was significant at 10 p. p. m., but not at 2 or 5 p. p. m. The means for the presence of copper and iron at 2 and 5 p. p. m. barely exceeded the values for iron alone. At 10 p. p. m., the results with iron alone appreciably exceeded those obtained in the presence of both iron and copper. The presence of copper alone gave but a slight increase over the values obtained in the absence of both copper and iron at 10 p. p. m., but more important increases at 2 and 5 p. p. m. This is illustrated in figure 1, where it appears that the presence of copper gave some increase in the absence of iron, but that copper was decidedly harmful when iron was present.

Interaction of Cu × Mn.—Significance was not obtained for interaction of copper and manganese at any of the three rates. It also appears from the means that the combinations are little better or poorer than might be anticipated by the simple addition of beneficial effects due to copper and manganese, as shown in the discussion above, when the results are analyzed for the presence or absence of either one of these singly. The graph shows that the addition of copper at 10 p. p. m. was without effect in the absence of manganese and was some what harmful when manganese was present.

Interaction of Cu× Zn.—At the three rates the mean weights in the presence of both zinc and copper slightly exceeded those obtained with zinc alone. Copper alone gave values identical with those obtained in the absence of both copper and zinc at 2 p. p. m., a slight increase at 5, and an appreciable decrease at 10. It will be noted that high significance for this interaction was reached at 2 and at 10 p. p. m., and that the interaction with R_2 was significant. This is somewhat unusual in that generally the results for 5 p. p. m. follow the linear component for 2 and 10 p. p. m. From the graph it will be noted that copper at 10 p. p. m. was distinctly harmful in the absence of zinc, but that it was beneficial when zinc was present. At 2 p. p. m. the results were similar to the extent that copper was beneficial in the presence of zinc. At 5 p. p. m. copper was beneficial in the absence and without effect in the presence of zinc.

[4] The scheme for graphic representation of comparisons, used in figures 1 and 2, was furnished by H. D. Barker.

Interaction of Fe × Mn.—Iron and manganese show no significant interactions at 2, 5, or 10 p. p. m. However, it will be noted that the interaction $R_2 \times Fe \times Mn$ reaches significance. At 2 p. p. m. the presence of manganese gives but slight increase over its absence when iron is either present or absent, the increase being relatively greater when iron is absent. At 10 p. p. m. the results are similar in that the presence of manganese is almost without effect when iron is present,

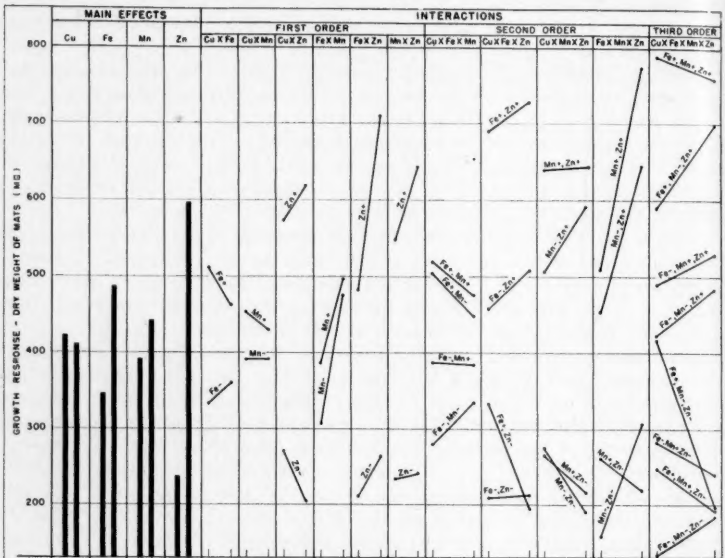

FIGURE 1.—Graphic representation of comparisons for main effects and interactions for experiment 108. The significance of an interaction is indicated by the degree of failure of the curves to extend in a parallel direction. The angle of convergence furnishes a measure for the relative importance of the various interactions, a wide angle being associated with a large contribution. The dry weight of mats produced was used to measure the growth response of *Phymatotrichum omnivorum* to the presence or absence indicated by plus (+) or minus (−) signs, at 10 p. p. m., of four elements in purified solutions.

and based upon comparative weights in the experiments at 2 and 10 p. p. m., the presence of manganese is of approximately equal value in the absence of iron. At 5 p. p. m. the results differ materially in that manganese is highly beneficial in the presence of iron, and in the absence of iron gives about the same proportional increase as occurred at the other rates.

Interaction of Fe × Zn.—This interaction is significant at all levels. At 2 p. p. m. the combined effect of iron and zinc gives about three and one-half times the value obtained in the presence of either iron or zinc

when the other member of the combination is absent. At 5 p. p. m. the mean weight with both elements present is not quite double that of zinc alone, but is nearly three times as large as that of iron alone, and the latter only slightly exceeds the values obtained when both are absent. At 10 p. p. m. the relationships parallel very closely those obtained at 5 p. p. m. Thus at 2 p. p. m. the results are materially different from those at 5 and 10 p. p. m. It appears likely that most of this difference is taken out with the interaction of R_1, which is highly significant, so that R_2 scarcely reaches the 5-percent requirement.

Interaction of Mn×Zn.—This interaction is significant at 5 and 10 p. p. m. At 2 p. p. m. the mean value in the presence of both elements only slightly exceeds that in the presence of zinc alone, and in the presence of manganese alone the mean value is little more than that obtained in the absence of both elements. At 5 p. p. m. the mean weight of mats obtained in the presence of manganese and zinc exceeds by as much as 25 percent the mean weight with zinc alone, while with manganese present alone, the mean weight is only slightly better than with both manganese and zinc absent, as was the case at 2 p. p. m. At 10 p. p. m., the value obtained with both manganese and zinc present appreciably exceeds that obtained with zinc alone, and again there is but slight difference between the values with manganese present alone and with both absent.

Second- and third-order interactions.—For the second-order interaction, in which rates are not involved, no significant differences are obtained except for Fe×Mn×Zn at 10 p. p. m. and in the combined analysis. At all three rates, the mean weight with iron, manganese, and zinc present shows that this is a particularly favorable combination. The mean weight with iron and manganese present and zinc absent does not exceed one-third of the preceding value. The value for iron and zinc about equals that for all three present at 2 p. p. m. and is about 25 percent less at 5 and 10 p. p. m. Manganese and zinc both present is the next best combination, although at 2 p. p. m. it only slightly exceeds some of the less favorable combinations. Zinc alone does not appear in favorable light for 2 p. p. m., but at 5 and 10 p. p. m. it is appreciably better than iron and is only slightly below manganese plus zinc. The interaction of Cu×Fe×Mn×Zn reaches high significance at 10 p. p. m. Figure 1 shows that in the absence of zinc the beneficial effects of iron are nullified when manganese or copper is added or when both are present, whereas, in the presence of zinc, iron not only reacts very favorably, but the additional presence of manganese or copper is beneficial although copper is slightly harmful if all three of the others are present. In considering the means for the interaction of Cu×Fe×Mn×Zn, it should be recalled that each of the means given here represents the results from five replicates at 2 and 10 p. p. m. and from four replicates at 5 p. p. m., and that the 2 p. p. m. experiment was harvested after 21 days' incubation, while the other experiments were harvested at 28 days.

Effect of Varying One Element at a Time, With Three Held Constant

To supplement certain of the results obtained in the preceding factorial experiments, four experiments, designated as 114, 115, 116,

and 117, were conducted in which the rate of one element was varied while the three other elements were held at a constant level of either 2 or 5 p. p. m. The element under consideration in a given experiment was studied as to absence or presence at 0, 2, 5, 10, 20, and 30 p. p. m., and each experiment was further divided to include two types of solution, purified and unpurified. For example, in experiment 114, manganese was the variable element, being used at 0, 2, 5, 10, 20, or 30 p. p. m. in both purified and unpurified solutions. Each kind of solution had received the three other elements (copper, iron, and zinc) at the rates of 2 and 5 p. p. m. of each. The factors under consideration in this experiment were kind of solution (2), level of three other elements (2), and rate of Mn application (6), giving a $2\times2\times6$ factorial, 24 treatments or combinations being required. As usual, the treatments were prepared on the basis of five flasks for each solution combination. In two of the experiments no contaminations occurred and all five replicates were included in the analyses. In the other two experiments each had one contaminated flask in one or more of the series so that only four replicates were included in the analyses, the fifth replicate, when available, being chosen at random and discarded.

From the outline just given of experiments 114 to 117 it will be seen that one of the objects was to test purified in comparison with unpurified solutions. As stated under discussion of methods, the formula for the unpurified solution requires that ferric chloride ($FeCl_3.6H_2O$) be added to the equivalent of 0.46 p. p. m. of iron. Hence, since it was impossible to test the standard unpurified solution at 0 p. p. m. for iron, the mean values of the four replicates obtained on the unpurified solution in experiment 116 are inserted in table 5 in their appropriate place, in parentheses. They are not included in the analysis of variance for experiment 116 in table 5, nor were these values used in obtaining the other mean values that are recorded for this experiment. It should also be borne in mind that in the other experiments (114, 115, and 117), where it is stated that iron and other elements are held constant at 2 or 5 p. p. m., this actually means that 2.46 and 5.46 p. p. m. are the correct values for iron.

Although these are separate experiments and in some respects are not directly related to each other, the results will be discussed on the basis of sources of variance, and the interpretations offered are based on the mean values and the analyses of variance of the respective experiments, as presented in table 5.

Kind of solution.—It will be seen from the mean values in table 5 that the purified solution gave very much higher results than the unpurified solution in all four experiments. These differences are highly significant.

Level of three elements held constant.—There was little difference in the results between 2 p. p. m. and 5 p. p. m. except where copper was the variable element (experiment 117), in which case the value at 5 p. p. m. was distinctly lower than at 2 p. p. m.

TABLE 5.—*Mean values and analysis of variance of mat weights of Phymatotrichum omnivorum in experiments in which one element was varied while others were held constant at 2 or 5 p. p. m. on two types of solution, with incubation at 28° C. for 28 days*

COPPER VARIABLE (EXPERIMENT 117)

Variance	Mean mat weight		Analysis of variance	
	Mean	Difference required for significance (odds 99:1)	D. F.	Mean square [1]
	Mg.	*Mg.*		
Kinds of solution		50.2	1	*1,534,923
Purified	726.1			
Unpurified	478.3			
Levels for 3 elements		50.2	1	*126,764
2 p. p. m.	637.8			
5 p. p. m.	566.6			
Rates of Cu		79.3	4	*182,657
0 p. p. m.	719.8			
2 p. p. m.	639.9			
5 p. p. m.	640.1			
10 p. p. m.	530.3			
20 p. p. m.	481.0			
Kinds × levels			1	7,566
	Mean mat weight with 3 elements at—			
	2 p. p. m.	5 p. p. m.		
Kinds of solution:	*Mg.*	*Mg.*		
Purified	753.0	699.2		
Unpurified	522.6	434.0		
Kinds × rates			4	*69,406
	Mean mat weight with Cu at—			
	0 p. p. m.	2 p. p. m.	5 p. p. m.	10 p. p. m.	20 p. p. m.		
Kinds of solution:	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*		
Purified	753.1	773.7	796.0	719.6	588.2		
Unpurified	686.4	506.1	484.2	341.0	373.9		
Levels × rates						4	*63,006
Levels at—							
2 p. p. m.	705.3	630.3	668.6	580.0	604.9		
5 p. p. m.	734.3	649.5	611.6	480.6	357.1		
Kinds × levels × rates						4	21,536
Purified, levels at—							
2 p. p. m.	745.0	746.7	828.4	708.3	736.6		
5 p. p. m.	761.3	800.6	763.5	730.9	439.7		
Unpurified, levels at—							
2 p. p. m.	665.6	513.8	508.8	451.7	473.2		
5 p. p. m.	707.3	498.3	459.7	230.2	274.6		
Error						80	9,037
Total						99	76,734

IRON VARIABLE (EXPERIMENT 116) [2]

Variance	Mean mat weight		Analysis of variance	
	Mean	Difference required for significance (odds 99:1)	D. F.	Mean square [1]
Purified solution:	*Mg.*	*Mg.*		
Levels for 3 elements		25.7	1	2,844
2 p. p. m.	726.7			
5 p. p. m.	742.1			
Rates of Fe		44.5	5	*78,927
0 p. p. m.	533.5			
2 p. p. m.	759.0			
5 p. p. m.	785.6			
10 p. p. m.	785.1			
20 p. p. m.	785.6			
30 p. p. m.	757.6			
Levels × rates			5	1,586

See footnotes at end of table.

TABLE 5.—*Mean values and analysis of variance of mat weights of Phymatotrichum omnivorum in experiments in which one element was varied while others were held constant at 2 or 5 p. p. m. on two types of solution, with incubation at 28° C. for 28 days*—Continued

IRON VARIABLE (EXPERIMENT 116)—Continued

Variance	Mean mat weight with Fe at—						Analysis of variance	
	0 p. p. m.	2 p. p. m.	5 p. p. m.	10 p. p. m.	20 p. p. m.	30 p. p. m.	D. F.	Mean square
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*		
Purified solution—Continued.								
Levels for 3 elements:								
2 p. p. m	545.9	745.9	777.5	789.8	759.7	741.4		
5 p. p. m	521.1	772.1	793.8	780.4	811.5	773.8		
(Unpurified solution:)								
(Levels at—								
2 p. p. m.)	(503.6)	(483.4)	(458.6)	(454.3)	(469.5)	(485.8)		
5 p. p. m.)	(470.7)	(457.1)	(437.8)	(454.1)	(453.7)	(431.0)		
Error							36	1,067
Total							47	9,443

MANGANESE VARIABLE (EXPERIMENT 114)

Variance	Mean mat weight		Analysis of variance	
	Mean	Difference required for significance (odds 99:1)	D. F.	Mean square[1]
	Mg.	*Mg.*		
Kinds of solution		26.4	1	*2,206,417
Purified	806.3			
Unpurified	503.1			
Levels for 3 elements		26.4	1	3,360
2 p. p. m	662.2			
5 p. p. m	647.3			
Rates of Mn		45.7	5	*10,040
0 p. p. m	641.9			
2 p. p. m	660.6			
5 p. p. m	648.1			
10 p. p. m	616.6			
20 p. p. m	673.6			
30 p. p. m	687.7			
Kinds × levels			1	*71,215

Variance	Mean mat weight with 3 elements at—		Analysis of variance	
	2 p. p. m.	5 p. p. m.	D. F.	Mean square[1]
	Mg.	*Mg.*		
Kinds of solution:				
Purified	786.6	826.1		
Unpurified	537.9	468.4		
Kinds × rates			5	*15,702

Variance	Mean mat weight with Mn at—						Analysis of variance	
	0 p. p. m.	2 p. p. m.	5 p. p. m.	10 p. p. m.	20 p. p. m.	30 p. p. m.	D. F.	Mean square[1]
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*		
Kinds of solution:								
Purified	827.1	826.4	766.5	726.1	826.4	865.7		
Unpurified	456.7	494.8	529.7	507.1	520.9	509.6		
Levels × rates							5	626
Levels at—								
2 p. p. m	651.4	665.1	657.4	633.7	679.8	685.9		
5 p. p. m	632.3	656.1	638.8	599.5	667.5	689.4		
Kinds × levels × rates							5	*9,249
Purified:								
Levels at—								
2 p. p. m	841.5	803.9	765.6	720.4	771.4	816.9		
5 p. p. m	812.6	849.0	767.4	731.8	881.4	914.5		
Unpurified:								
Levels at—								
2 p. p. m	461.3	526.4	549.2	547.0	588.2	555.0		
5 p. p. m	452.1	463.2	510.2	467.3	453.6	464.3		
Error							72	2,382
Total							95	27,712

See footnotes at end of table.

TABLE 5.—*Mean values and analysis of variance of mat weights of Phymatotrichum omnivorum in experiments in which one element was varied while others were held constant at 2 or 5 p. p. m. on two types of solution, with incubation at 28° C. for 28 days*—Continued

ZINC VARIABLE (EXPERIMENT 115)

Variance	Mean mat weight						Analysis of variance	
	Mean						Difference required for significance (odds 99:1)	D. F.	Mean square [1]
	Mg.						*Mg.* 20.3	1	*2,923,472
Kinds of solution									
Purified	686.5								
Unpurified	374.4								
Levels for 3 elements							20.3	1	2,365
2 p. p. m.	534.9								
5 p. p. m.	526.0								
Rates of Zn							35.2	5	*562,326
0 p. p. m.	188.9								
2 p. p. m.	616.4								
5 p. p. m.	605.3								
10 p. p. m.	586.8								
20 p. p. m.	588.1								
30 p. p. m.	597.1								
Kinds × levels								1	*14,172
	Mean mat weight with 3 elements at—								
	2 p. p. m.						5 p. p. m.		
Kinds of solution:	*Mg.*						*Mg.*		
Purified	680.1						692.9		
Unpurified	389.7						359.0		
Kinds × rates								5	*114,247
	Mean mat weight with Zn at—								
	0 p. p. m.	2 p. p. m.	5 p. p. m.	10 p. p. m.	20 p. p. m.	30 p. p. m.			
Kinds of solution:	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*			
Purified	204.9	770.8	775.5	744.0	788.1	835.7			
Unpurified	172.9	462.1	435.1	429.5	388.1	358.5			
Levels × rates								5	*11,854
Levels at—									
2 p. p. m.	181.2	601.9	579.6	598.1	616.5	632.0			
5 p. p. m.	196.7	631.0	631.0	575.5	559.7	562.2			
Kinds × levels × rates								5	*11,544
Purified:									
Levels at—									
2 p. p. m.	175.6	717.6	715.7	757.8	836.9	877.0			
5 p. p. m.	234.3	824.0	835.3	730.2	739.3	794.5			
Unpurified:									
Levels at—									
2 p. p. m.	186.8	486.2	443.5	438.3	396.0	387.1			
5 p. p. m.	159.0	437.9	426.6	420.8	380.1	329.9			
Error								96	1,794
Total								119	55,564

[1] Asterisk (*) indicates that contributor is highly significant (odds 99:1).

[2] All unpurified solutions contained 0.46 p. p. m. of iron, as called for in the preparation of the solution, plus whatever iron is indicated for the various treatments. It was felt that the additional 0.46 p. p. m. in the unpurified solution would be of little consequence in restratifying the data as shown for experiments 117, 114, and 115 for comparing levels of iron at 2 and 5 p. p. m. Since, however, there could be no zero rates of iron with this standard unpurified solution it seemed unwise to restratify the data for rates of iron, or nteractions with rates, for experiment 116, when iron was the variable element. Hence, the analysis of variance and all data for experiment 116, except those given in parentheses, refer to purified solutions only.

Rate of application of variable element.—For copper (experiment 117) it will be noted that the result in its complete absence was very much superior to that obtained when it was present. The mean values for copper present at 2 or 5 p. p. m. were about equal in value; 10 p. p. m.

caused a considerable drop, and 20 p. p. m. a further drop; copper at 30 p. p. m. behaved in such an erratic manner that the results were not considered reliable, and accordingly they have not been included. For iron (experiment 116), which included only the purified solution, the result obtained in its presence was distinctly superior to that obtained in its absence. There was little difference between rates ranging from 2 to 30 p. p. m. The presence of manganese (experiment 114) at various rates was slightly superior to its absence, except at 10 p. p. m. The differences between rates barely reached significance at the 1-percent requirement. For zinc there was a remarkable difference between presence and absence, values with the latter being about one-third of those with the former. No appreciable difference occurred with zinc in rates from 2 to 30 p. p. m.

Kind×*level.*—In experiment 117, with copper variable, the purified solution at 2 p. p. m. was distinctly superior to the 5 p. p. m.; the same relationship between 2 and 5 p. p. m. existed in the unpurified solution. For experiment 114, with manganese variable, 5 p. p. m. was appreciably better than 2 p. p. m. in the purified solution. The reverse was true of the unpurified solution, a highly significant *F* value for kind×level being found in experiment 114 (see table 5). There was little difference between the results at 2 and 5 p. p. m. in the purified solution for zinc, although in the unpurified solution 2 p. p. m. was slightly better. The interaction was highly significant in this experiment (115).

Kind×*rate.*—At 0 to 5 p. p. m., copper in the purified solution gave very excellent growth; at 10 p. p. m. growth was only slightly inferior; but at 20 p. p. m. it was decidedly poorer. In the unpurified solution the amount of growth in general decreased with increasing concentrations of copper. It is important to note again the very considerable increase in growth when there was no copper in the unpurified solution. The significant interaction is probably explained by the fact that depression of growth with the increasing concentrations of copper commenced sooner and progressed more rapidly in the unpurified solution than in the purified. Manganese gave very fine growth in the purified solution at all rates. In the unpurified solution, for which mat weight was less than two-thirds that of the purified solution, concentration seemed also to have little effect, except that growth averaged about 50 mg. less when manganese was absent than when it was present. This interaction was significant at the 1-percent requirement. For experiment 115, with zinc variable, growth was very poor in both the purified and the unpurified solution in the absence of zinc. In the purified solution the average mat weight tended to increase with increasing concentration, whereas in the unpurified solution the rate of growth decreased at a fairly regular rate with increasing concentration. The interaction was significant.

Level×*rate.*—In experiment 117, the mat weights were decidedly better in the absence of copper than in its presence for both the 2 p. p. m. and the 5 p. p. m. levels of other elements. Increasing concentrations of copper from 2 to 20 p. p. m. seemed to be without material effect at the 2 p. p. m. level, whereas at the 5 p. p. m. level of other elements the decrease in mat weight was fairly rapid with increase of concentrations. In the purified solution (experiment 116), which is the only solution included for iron, rate of application removed most

of the variance. Interaction was not significant. For manganese the condition was very similar to that for iron, rate removing most of the variance. For zinc, optimum growth was apparently reached at the highest concentrations when the level of other elements was held at 2 p. p. m., although growth with zinc at 30 p. p. m. was not very much greater than with zinc at 2 p. p. m. When the other elements were held at 5 p. p. m., the optimum appeared to be reached at 2 and 5 p. p. m. of zinc, 10, 20, and 30 p. p. m. of zinc giving lower results which did not differ greatly from one another.

Kind×*level*×*rate.*—Although the highest order interaction did not reach significance at the 1-percent requirement for experiment 117, several interesting comparisons are apparent. With copper absent in the unpurified solution and the other elements at 5 p. p. m., the mean mat weight for five replicates was over 700 mg., and with the other elements present at 2 p. p. m. (and copper absent) the average was only about 40 mg. less. The decrease with additional amounts of copper was quite rapid in the unpurified solution when the other elements were held constant at 5 p. p. m. One other feature of interest regarding copper is that, when the other elements were present at 2 p. p. m. in the purified solution, as much as 20 p. p. m. of copper seemed to do little harm. When the other elements were at 5 p. p. m. in the purified solution the depression in mat weight was quite sharp at 20 p. p. m. of copper.

Although the results for iron in the unpurified solution were not included in the means or the analysis shown in table 5 (experiment 116), the values are included in parentheses because they appeared to be of interest in connection with the copper and iron relationship. These results are interpreted to indicate that when iron is added to the amount of only 0.46 p. p. m. in the unpurified solution the growth obtained is better than when iron is present in larger amounts. Increasing amounts of iron in purified solution appeared to do no harm. These results indicate some unexplained incompatibility of copper and iron in the unpurified solution. In experiment 108 it was shown that slightly deleterious effects occurred when copper and iron at 10 p. p. m. were present, even in the purified solution.

Interactions were significant for manganese and zinc (experiments 114 and 115). In the purified solution manganese did not appear to be beneficial, nor did increasing amounts of manganese appear to do harm when the other elements were at 2 or 5 p. p. m. The highest mean value obtained was with manganese at 30 p. p. m. and the other elements at 5 p. p. m. In the unpurified solution, growth in the presence of manganese was somewhat better than in its absence when the other elements were at 2 p. p. m. The presence or absence of manganese had little appreciable effect upon growth in the unpurified solution with the other elements at 5 p. p. m. Increasing amounts of zinc up to 30 p. p. m. appeared to be beneficial in the purified solution with other elements at 2 p. p. m.; with other elements at 5 p. p. m. optimum growth with zinc occurred at 2 to 5 p. p. m. In the unpurified solution with other elements at 2 p. p. m., the optimum concentration of zinc was 2 p. p. m. with rather sharp decreases above 10 p. p. m. A similar condition existed with the other elements at 5 p. p. m. In all four treatments, where zinc was absent growth was poor.

Effect of Varying Iron and Zinc With Manganese Held Constant

Several of the problems involved in determining the best type of medium for optimum growth of the root rot organism were solved in the foregoing experiments (114–117), when it became evident that it would not be necessary to resort to the laborious process of purification since excellent growth could be obtained on the unpurified solution when copper was omitted. Under the heading "Kind of solution" (p. 149), it was pointed out that the mean for the purified solution ex-

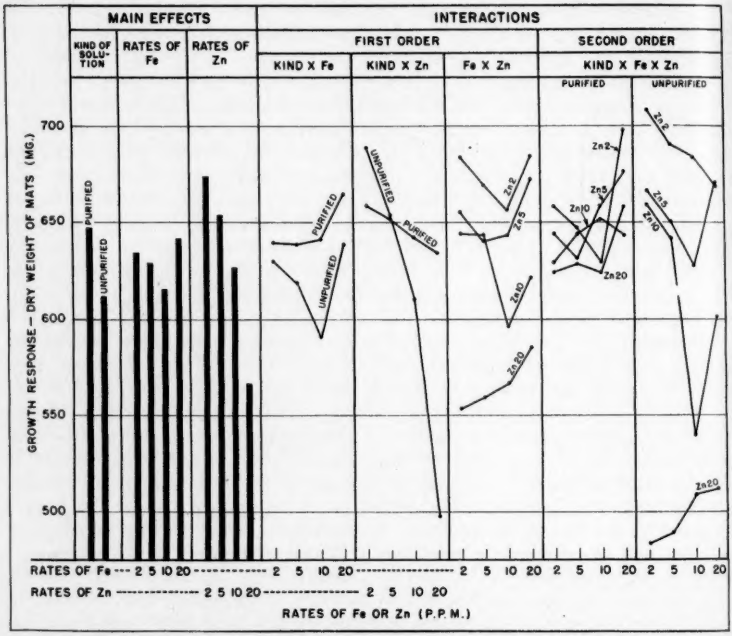

Figure 2.—Graphic representation of comparisons for main effects and interactions for experiment 119. Curves representing second-order comparisons are divided into two groups and compared side by side instead of being superimposed on each other as in figure 1. Nevertheless, curves for the purified solution are nearly on top of each other as shown. The dry weight of mats produced was used to measure the response of *Phymatotrichum omnivorum* to varying rates of iron and zinc in purified and unpurified solutions. Copper was omitted and manganese was held constant at 2 p. p. m.

ceeded by at least 50 percent that for the unpurified solution. Somewhat similar relationships prevailed in all previous experiments. The results with copper absent from the unpurified solution indicated how this might be corrected. It was demonstrated that the rate of manganese application was of little importance and that the presence of manganese gave results only slightly superior to its absence. Hence in the present experiment copper was omitted and manganese was held constant at 2 p. p. m. Iron and zinc were used in a factorial design at four levels, 2, 5, 10, and 20 p. p. m. The unpurified solution in this experiment differed from that in the preceding experiments (114–117) in that ferric chloride ($FeCl_3.6H_2O$) was omitted in the

preparation of the solution, hence iron would be present only as impurities or as added later in the various treatments. As in the preceding experiment, five flasks of each treatment were prepared but only four were considered in the analysis, the fifth being discarded in each case by random choice if no contaminations developed. The results of this experiment are presented in table 6 and in figure 2.

Kind of solution.—The growth in purified solution exceeded that in the unpurified solution by less than 35 mg. However, since these means were derived from 64 replicates each, this difference reaches high significance.

TABLE 6.—*Effect of varying iron and zinc at four rates, with manganese held constant (experiment 119)*

Variance	Mean mat weight		Analysis of variance	
	Mean	Difference required for significance (odds 99:1)	D. F.	Mean square[1]
	Mg.	*Mg.*		
Kinds of solution		16.94	1	*37,565
Purified	646.59			
Unpurified	612.32			
Rates of iron application		23.95	3	3,798
Rates of zinc application		23.95	3	*69,349
	Mean mat weight with Fe variable	Mean mat weight with Zn variable		
Rates of iron or zinc:	*Mg.*	*Mg.*		
2 p. p. m.	633.93	672.88		
5 p. p. m.	627.61	652.95		
10 p. p. m.	615.31	626.20		
20 p. p. m.	640.97	565.79		
Kinds of solution×rates of Fe			3	4,117
Kinds of solution×rates of Zn			3	*41,581
Kinds×Fe or Zn:				
Purified, rate at—				
2 p. p. m.	638.95	658.17		
5 p. p. m.	637.60	652.58		
10 p. p. m.	640.80	641.93		
20 p. p. m.	668.99	633.68		
Unpurified, rate at—				
2 p. p. m.	628.90	687.59		
5 p. p. m.	617.62	653.32		
10 p. p. m.	589.83	610.48		
20 p. p. m.	612.95	497.91		
Rates of Fe×rates of Zn			9	1,664

	Mean mat weight with zinc at—					
	2 p. p. m.	5 p. p. m.	10 p. p. m.	20 p. p. m.		
Fe at—	*Mg.*	*Mg.*	*Mg.*	*Mg.*		
2 p. p. m.	682.84	655.34	644.03	553.50		
5 p. p. m.	668.58	640.30	643.00	558.56		
10 p. p. m.	656.31	643.14	595.69	566.11		
20 p. p. m.	683.80	673.01	622.09	584.99		
Kind×Fe×Zn					9	2,625
Purified, Fe at—						
2 p. p. m.	658.08	644.28	629.18	624.28		
5 p. p. m.	647.48	630.83	644.28	627.83		
10 p. p. m.	628.98	659.08	650.98	624.18		
20 p. p. m.	698.15	676.13	643.28	658.43		
Unpurified, Fe at—						
2 p. p. m.	707.60	666.40	658.88	482.73		
5 p. p. m.	689.68	649.78	641.73	489.30		
10 p. p. m.	683.65	627.20	540.40	508.05		
20 p. p. m.	669.45	669.90	600.90	511.55		
Error					96	1,331
Total					127	4,413

[1] Asterisk (*) indicates that *F* value obtained is highly significant (odds 99:1).

Rates of Fe application.—The mean mat weights with iron at concentrations of 2, 5, and 20 p. p. m. were not significantly different. For some reason difficult to explain on the basis of available data, 10 p. p. m. gave comparably low yields, whereas 20 p. p. m. gave an average mat weight which slightly exceeded that obtained at 2 p. p. m.

Rates of Zn application.—The optimum concentration for zinc was clearly at 2 p. p. m. in the combined means obtained from the different kinds of solution, and from various combinations with iron. Increasing concentration resulted in steady decreases.

Kind of solution×*Fe.*—With iron it will be noted that the purified solution gave a better performance than the unpurified solution, although at 2 p. p. m. the difference was small. In the purified solution, there was little difference between 2, 5, and 10 p. p. m. while 20 p. p. m. gave a considerably higher mean mat weight. In the unpurified solution, results with iron at 2 p. p. m. appeared to be optimum, with iron at 5 p. p. m. somewhat lower, and with iron at 10 p. p. m. distinctly inferior. Here again the peculiar increase with 20 p. p. m. mentioned above will be noted. Since highly significant interactions were obtained in this experiment and since one of the primary objects was to clarify the intricate relationships of these two most important elements (iron and zinc), it is felt that the preceding tables and analyses of variance could be more easily interpreted by the aid of the graphs presented in figure 2.

Kind of solution×*Zn.*—In the purified solution the different rates of zinc were comparatively unimportant. However, there was a slight downward trend with increasing concentration. In the unpurified solution, the values with zinc at 2 p. p. m. exceeded all values obtained in the purified solution, and there was a very rapid and regular decrease from 2 to 10 p. p. m.; from 10 to 20 p. p. m. the decrease was even greater. This resulted in a very high *F* value for interaction.

Fe×*Zn.*—At all concentrations of iron, 2 p. p. m. of zinc gave the best average results, and increasing concentrations of zinc resulted in fairly regular average decreases. Or, considering the curves for zinc, it will be noted that 2 and 20 p. p. m. of iron gave the best general results, except when zinc was at 20 p. p. m., with a particularly unfavorable combination for zinc at 2 and 10 p. p. m. However, since these differences were brought out with rates of application, there is no significant interaction for rates of iron×rates of zinc.

Kind of solution×*Fe*×*Zn.*—This is not a significant interaction. The behavior of the four rates of iron application respond very much in the same manner with increasing concentration of zinc, particularly in the unpurified solution. In the purified solution, the differences due to increasing concentration of zinc are small and somewhat irregular, but again their trend is downward.

From a practical point of view, the most important features of the above experiment were as follows: (1) The results suggested in experiments 114 to 117, that the unpurified solution would serve satisfactorily for optimum growth, were confirmed; actually the highest mat weight recorded was with the unpurified solution when iron, zinc, and manganese were present at 2 p. p. m. (2) The concentration of iron does not seem to be so important as the concentration of zinc. (3) The unpurified solution is decidedly more sensitive to increasing concentrations of zinc than is the purified solution. (It will be recalled from experiment 117 that the unpurified solution was also exceedingly

sensitive to copper application.) (4) A peculiar but unexplained response with iron application was observed in both the purified and unpurified solution. In the purified solution, 2, 5, and 10 p. p. m. gave about equal results but 20 p. p. m. gave a decided increase, and in the unpurified solution 20 p. p. m. gave an increase over 10 p. p. m., the most unfavorable combination. A somewhat similar relationship was noted with manganese in experiment 114 in the purified solution. (5) While the distinctions between 2 and 5 p. p. m. are not great and on the purified solution it has been almost impossible to decide whether 2 or 5 p. p. m. provided optimum growth conditions, it now seems that in the unpurified solution the best concentration for optimum conditions may definitely be stated as not more than a 0–2–2–2 concentration of copper, iron, manganese, and zinc; subsequent experiments have been based on this formula.

Effect of Iron, Manganese, and Zinc on Different Isolates

In some subsequent experiments on fungus nutrition it appeared that the presence of iron was not quite so important as the preceding experiments had indicated. This gave rise to speculation as to whether these results were caused by some uncontrolled factor, whether the fungus was losing some of its vegetative vigor, or whether different isolates might fail to respond uniformly to the treatments with the above elements that had been found necessary for optimum growth. Accordingly, the studies were extended to include additional isolates of the fungus collected during the summer of 1938 from various root rot-infested fields of central Texas. From the newly isolated cultures of *Phymatotrichum omnivorum* seven isolates were chosen for comparative studies with isolate 28, which had been isolated in 1937, and on which the preceding studies had been made.

Preliminary comparisons on solid media and on the unpurified nutrient solution indicated that the eight isolates differed in the amount of growth produced on the several substrates. For the more critical experiments to determine the response of isolates to the addition of certain elements, the number of isolates was reduced to four, isolate 28 being used as the standard and isolates 4, 5, and 7 being compared with it. Two experiments were conducted in the factorial design, the variable factors being isolates (4), treatments (5), and time of harvest (2 or 4). The treatments included were (0) no elements added to the unpurified solution, (A) zinc added, (B) iron and zinc added, (C) manganese and zinc added, and (D) iron, manganese, and zinc added, the elements, whether singly or in combination, being added at the rate of 2 p. p. m. each to the unpurified solution. In the first of these two experiments, designated as N–22, the mats were harvested at 14, 18, 22, and 26 days after inoculation, three replicates of each combination being used. The second experiment (N–26) was based upon harvests at 14 and 22 days, replicates four. The incubation was at 28° C. in both experiments. The response of the four isolates to the several treatments was much the same in both experiments, and an analysis of differences between the two experiments yielded no values which approached significance even at the 5-percent level, indicating that with the harvests at 14 and 22 days, common in both experiments, the two experiments might be considered as one and analyzed as such. The results of the 14- and 22-day harvests of the two experiments were, therefore, brought together in one analysis,

thus giving seven replicates. The summary of this analysis and the mean weights of the various combinations are presented in table 7. Since the analysis of differences at 14 and 22 days for experiments N–22 and N–26 suggested that the results might be analyzed together, it is assumed that conclusions may be drawn from either experiment as well as from the combined analysis. In N–22 there were four dates of harvest so that the growth curves obtained are more complete than could be obtained from N–26 or the combined analysis. Accordingly it seemed desirable to present these growth curves (fig. 3) and to base the interpretation and discussion of the results on the graph and the table of combined results.

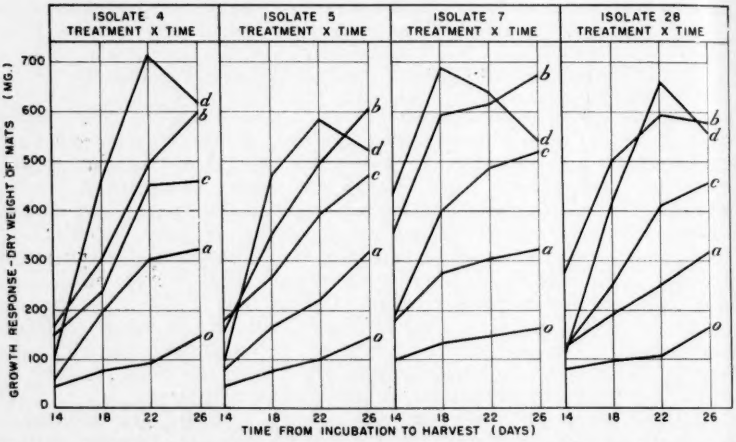

FIGURE 3.—Graphic representation of comparisons for experiment N–22. Dry weight of mats produced is used to measure the effect of several combinations of iron, manganese, and zinc at 2 p. p. m. on the growth of four isolates of *Phymatotrichum omnivorum* in unpurified solutions at 28° C. For each isolate treatment×time (or rate of growth) may be considered singly as a first-order interaction or the figure as a whole may be considered to represent the second-order interaction, isolate×treatment×time. *0*, Check (all three elements omitted); *a*, zinc alone added; *b*, iron and zinc added; *c*, manganese and zinc added; *d*, iron, manganese, and zinc added.

Isolates.—Of the four isolates, No. 5 gave a comparatively low mat weight. This difference from the other three was highly significant. Isolates 4 and 28 gave similar mean mat weights, which differed significantly from the slow-growing isolate 5 and the rapid-growing isolate 7.

Treatments.—All treatments differed significantly from one another. The mean mat weight was greatest in treatment D (Fe, Mn, Zn), followed by treatment B (Fe, Zn), treatment C (Mn, Zn), treatment A (Zn), and treatment 0 (none added).

Time×isolate.—The four isolates responded in a similar manner to the differences in time of harvest when measured at 14 and 22 days only, and the mean square obtained in the combined analysis of variance scarcely exceeds that of error. However, this was not true when experiment N–22 was analyzed separately, and it is believed

that this would not have been the case had N–26 been harvested at four dates or times. Figure 3 shows that all treatments do not reach their peak at the same time, nor do all isolates respond equally well to treatments, particularly in the early stages of development. These differences may escape detection when the harvest is made at 14 and 22 days only.

TABLE 7.—*Mean values and analysis of variance for reactions of four isolates of Phymatotrichum omnivorum to combinations of iron, manganese, and zinc*

[Experiments N–22 and N–26 combined for yield at 14 and 22 days]

Variance	Mean mat weight					Analysis of variance	
	Mean		Difference required for significance (odds 99:1)			D. F.	Mean square [1]
	Mg.		*Mg.*				
Time of harvest			18.30			1	*4,112,316
14 days	170.09						
22 days	412.47						
Isolates			25.87			3	*95,392
No. 4	289.87						
No. 5	247.13						
No. 7	337.51						
No. 28	290.62						
Treatments			28.95			4	*917,603
0	124.27						
A	199.99						
B	392.52						
C	311.79						
D	427.83						
Time×isolates						3	4,981
	Mean mat weight with isolate—						
	No. 4	No. 5	No. 7	No. 28			
Time of harvest:	*Mg.*	*Mg.*	*Mg.*	*Mg.*			
14 days	158.07	131.19	224.43	166.68			
22 days	421.67	363.07	450.59	414.55			
Time×treatments						4	*257,713
	Mean mat weight with treatment—						
	0	A	B	C	D		
Time of harvest:	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*		
14 days	88.56	126.66	215.37	188.55	231.31		
22 days	159.98	273.31	569.67	435.04	624.35		
Isolates×treatments						12	*13,580
Isolate—							
No. 4	117.01	214.54	341.01	319.90	456.90		
No. 5	98.22	163.19	317.35	284.49	372.41		
No. 7	144.35	227.98	490.26	341.21	483.72		
No. 28	137.51	194.24	421.46	301.58	398.29		
Time×isolates×treatments						12	5,811
14 days, isolate—							
No. 4	79.57	123.01	175.99	187.73	224.06		
No. 5	68.33	86.27	149.81	176.69	174.87		
No. 7	111.93	164.83	290.27	215.74	339.36		
No. 28	94.43	132.53	245.41	174.04	186.97		
22 days, isolate—							
No. 4	154.46	306.06	506.03	452.07	689.74		
No. 5	128.11	240.11	484.89	392.29	569.96		
No. 7	176.77	291.13	690.26	466.69	628.09		
No. 28	180.59	255.94	597.51	429.11	609.61		
Error						240	3,468
Total						279	36,486

[1] Asterisk (*) indicates that contributor is highly significant (odds 99:1).

Time×treatment.—It will be seen that with treatment 0 the mean mat weight was not doubled during the period from 14 to 22 days, whereas with treatments B and D the weights had increased more than two and one-half times during the same period and high significance was obtained.

Isolate×treatment.—This interaction was highly significant, owing largely to the fact that strain 4 responded unusually well to treatment D (Fe, Mn, Zn), whereas, with isolates 7 and 28 at 22 days, treatments B and D were not very different from each other. Treatment C is only slightly inferior to treatment B for isolate 4, but in relation to the better treatments it gives comparatively poor results for isolates 7 and 28. It is interesting to note from figure 3 that for the poorer treatments, C, A, and 0, the final mat weights at 22 days from each such treatment are not actually very different for the four isolates.

Time×isolate×treatment.—This interaction does not approach significance in the combined analysis. However, in the analysis of N–22 based upon four times of harvest, a significant value was obtained. The explanation of this has been pointed out in the discussion of the interaction time×isolate.

In conclusion, the most important features of the foregoing test may be summarized with the following statements. On the basis of mean mat weights attained with different treatments, isolates 4 and 28 are classed together, intermediate in position between isolate 7, or a significantly higher group, and isolate 5, of a lower group. Although, isolates 7 and 28 are in different subgroups on the basis of mean weights it has been pointed out that these two isolates react similarly in their response to the various treatments. It is of possible significance that isolates 7 and 28 were originally obtained from the same location in a single root rot spot in an otherwise root rot-free cotton field. The collections were made in 2 successive years. Their differential response to treatments suggests their identity, despite the fact that their growth rate was different. The latter may possibly be explained by the fact that the slower growing isolate had been carried in culture for a year longer than the faster growing one. It has been commonly observed with other fungi that differences may be detected in vegetative vigor following continued growth on artificial media. Consequently it was not so surprising to find that the various isolates differed in vegetative vigor or growth rate as it was to discover that isolates of *Phymatotrichum omnivorum* responded differentially to the various combinations of iron, manganese, and zinc.

FURTHER EXPERIMENTS WITH OTHER TRACE ELEMENTS

Earlier experiments have failed to demonstrate beneficial results from the addition of certain of the trace elements (aluminum, boron, etc.) to the purified solution when added to yield concentrations ranging from 1 to 10 p. p. m. Although these elements alone failed to give increases in mat weight, it was considered possible that if they were used in combination with iron, manganese, and zinc a further response in mat weight might be obtained. Accordingly, to the purified solution plus iron, manganese, and zinc (at 2 p. p. m. each) the elements were added singly to portions of the solution to give concentrations of 2, 4, or 10 p. p. m. The various treatments were set up in quadruplicate and were harvested after 21 days' incubation at 28° C. The results of this experiment are summarized in table 8.

With regard to the differences between the elements, it may be stated that a preliminary analysis of variance was made for each rate in order to establish the differential response of the fungus to the 11 elements under consideration when iron, manganese, and zinc were present. It was found that more than 58 mg. but less than 59 mg. was required for highly significant differences between the means of any 2 elements at both 2 and 4 p. p. m. None of the elements was significantly better or poorer than the check at 2 p. p. m., nor at 4 p. p. m. with the exception of nickel, which significantly depressed growth, and aluminum, which caused an increase over the check that approached significance. However, at both 2 and 4 p. p. m. certain of the better treatments could be differentiated from the poorer. It did not seem worth while to present the details of the separate analyses since the combined analysis at the 3 rates (table 9) seemed to bring out all the essential facts mentioned above, plus the additional information concerning rates and interactions of rate × element.

TABLE 8.—*Response of Phymatotrichum omnivorum to the addition of various elements to a purified solution containing Fe, Mn, and Zn, incubated at 28° C. for 21 days (experiment 120)*

Element [1]	Mean mat weight at—			Mean [2]
	2 p. p. m.	4 p. p. m.	10 p. p. m.	
	Mg.	*Mg.*	*Mg.*	*Mg.*
Aluminum	605.2	667.0	633.7	635.32
Fluorine	616.0	642.2	638.4	632.24
Boron	611.8	634.2	601.0	615.63
Mercury	617.7	599.9	618.5	612.04
Silicon	589.4	614.4	597.4	600.42
Iodine	602.4	616.9	560.8	593.34
Cadmium	570.9	591.7	589.1	583.89
Molybdenum	553.7	550.6	578.1	560.79
Cobalt	603.9	570.4	43.3	405.86
Nickel	575.5	535.9	14.9	375.43
Mean [2]	594.65	602.34	487.50	

[1] The following results, which were not included in table 8 nor in the analysis given in table 9, were secured with the elements or the checks indicated: Lithium at 4 p. p. m. gave 583.5 mg.; 10 p. p. m., 572.2 mg. Check A, with Fe, Mn, and Zn at 2 p. p. m., but other trace elements absent, gave 608.9 mg. Check B, with Fe, Mn, and Zn at 2 p. p. m. and also lithium and all of the 10 trace elements listed in the above table at 2 p. p. m., gave 577.3 mg.

[2] Difference required for significance (odds 99:1): Elements=34.51 mg.; Rates=18.90 mg.

For the analysis only 10 of the 11 elements were considered, since the lithium series was not complete for all 3 rates. A subsequent test with lithium showed no material difference for rates at 2, 4, and 10 p. p. m. Comparisons were made of the 10 elements at the 3 rates, and it will be observed that there was little difference between the 2 lowest rates, the mean for 2 p. p. m. being 594.65 mg. and for 4 p. p. m. 602.34 mg. The addition of cobalt or nickel at 10 p. p. m. resulted in almost complete inhibition of growth, whereas the other elements behaved very much as at 2 and 4 p. p. m. The depression of growth with the addition of cobalt or nickel at the rate of 10 p. p. m. resulted in a highly significant difference in the comparison of the mean of the rates of 2 plus 4 p. p. m. with 10 p. p. m.

In the analysis, which includes all three rates, it is also evident that no element is distinctly superior to the check, but certain group

responses can be identified. In order to emphasize this grouping, the 9 degrees of freedom for elements were broken down in the manner explained in footnote 2 of table 9.

TABLE 9.—*Analysis of variance for experiment 120*

Variance	D. F.	Mean square	*F*		
			Found	Required	
				1 percent	5 percent
Total	119	22,053.9345			
Rates: [1]					
R_1	1	1,183.4911	1.15	6.92	3.95
R_2	1	328,552.6079	318.46	6.92	3.95
Elements: [2]					
E_1	1	5,554.0838	5.38	6.92	3.95
E_2	1	3,201.6600	3.10	6.92	3.95
E_3	1	300.3338			
E_4	1	77.4004			
E_5	1	56.7338			
E_6	1	7,225.0669	7.00	6.92	3.95
E_7	1	4,772.0408	4.63	6.92	3.95
E_8	1	36,875.4401	35.74	6.92	3.95
E_9	1	875,700.2175	848.80	6.92	3.95
Rates×elements	18	70,448.1485	68.28	2.20	1.75
Error	90	1,031.6939			

[1] Comparisons between rates:
R_1=2 p. p. m. compared with 4 p. p. m.
R_2=Mean of rates 2 and 4 p. p. m. compared with 10 p. p. m.
[2] Comparisons between elements:
E_1=Co compared with Ni.
E_2=Cd compared with Mo.
E_3=Si compared with I.
E_4=B compared with Hg.
E_5=Al compared with F.
E_6=Mean effect of Si and I compared with mean effect of Cd and Mo.
E_7=Mean effect of Al and F compared with mean effect of B and Hg.
E_8=Mean effect of Al, F, B, and Hg compared with mean effect of Si, I, Cd, and Mo.
E_9=Mean effect of Al, F, B, Hg, Si, I, Cd, and Mo compared with mean effect of Co and Ni.

It will be noted that cobalt and nickel (comparison E_1) both inhibit growth almost completely at 10 p. p. m., nickel slightly more than cobalt. However, the difference in the extent to which growth was depressed by these elements does not reach significance at the 1-percent requirement. As is shown by the mean square values obtained for E_2, E_3, E_4, and E_5, for comparison of cadmium with molybdenum, silicon with iodine, boron with mercury, and aluminum with fluorine, respectively, the individual differences are insignificant. E_6, showing the mean effect of silicon and iodine as compared with cadmium and molybdenum, reaches high significance, while E_7, comparing the mean effect of aluminum and fluorine with boron and mercury, exceeds the 5-percent but does not reach the 1-percent requirement. In E_8 the four elements aluminum, fluorine, boron, and mercury, which consistently gave higher values than the check series (608.9 mg.), are compared with the next four elements, silicon, iodine, cadmium, and molybdenum. A highly significant *F* value is obtained for this comparison. The comparison of the mean effect of the eight elements considered in E_8 with the mean of cobalt and nickel yields a high *F* value, as might be expected. For the interaction of rates× elements, a high *F* value is obtained, and a partial explanation of this is afforded by the fact that eight of the elements maintain a moderately constant mat weight at all rates as contrasted with the marked depression of mat weights with cobalt and nickel with increases in

concentration. Although the nutrient solution was highly favorable for growth of the organism, cobalt and nickel showed the same high degree of toxicity at 10 p. p. m. as was shown in earlier experiments on the purified solution to which iron, manganese, and zinc had not been added following purification. However, cobalt does not show in this experiment the severely depressing effect at 4 p. p. m. that resulted in the earlier experiments.

A similar experiment was conducted on the unpurified solution with iron, manganese, and zinc, added at the rate of 2 p. p. m. each, and with the other elements added singly at the same rate. The mean weights, based upon five replicates of the various treatments, were as follows:

Element added:	*Mg.*	Element added:	*Mg.*
B	732. 4	Mo	618. 9
Al	729. 9	F	610. 1
Cd	718. 3	Hg	587. 7
Si	704. 8	Li	568. 5
Control	688. 1	Co	553. 3
Ni	674. 2	I	526. 6

The difference required between means for significance at odds of 99 : 1 was 138.6 mg. No significant increases in mat weight over the control were obtained through the addition of these elements, but it is of interest to note that boron and aluminum rank first and second, while in the preceding experiment they ranked third and first, respectively. Decreases in mat weight were obtained with the addition of a number of the elements, but only with iodine was the difference highly significant, although cobalt closely approached the requirement.

On the basis of these experiments it may be concluded that the addition of the trace elements aluminum, boron, etc., to purified or unpurified solutions containing iron, manganese, and zinc fails to give significant increases in mat weight over that obtained with the iron-manganese-zinc combination alone. These results suggest that the trace elements in the former group are not essential to *Phymatotrichum omnivorum* or that they are furnished in sufficient quantities as impurities, either in the solution or in the inoculum piece, to insure good growth of the fungus.

SUMMARY AND CONCLUSIONS

In an effort to develop the most satisfactory medium possible for certain fundamental studies with the root rot organism (*Phymatotrichum omnivorum*), several experiments were made to find what elements are needed for optimum growth and the proper proportion of each.

Of the four trace elements copper, iron, manganese, and zinc, which were studied intensively in these experiments, it appeared that at least three are essential for the optimum growth of *Phymatotrichum omnivorum*. Preliminary studies revealed the surprising fact that a very much higher concentration of these elements was required than had been found to be the case for several of the organisms studied by other workers.

Other trace elements—aluminum, boron, cadmium, cobalt, fluorine, iodine, lithium, mercury, molybdenum, nickel, and silicon—were found to be nonessential for optimum growth under the conditions employed in these studies. This was true whether iron, manganese,

and zinc were present or absent. It may be noted in passing that aluminum and boron were fairly consistent in giving a slight increase in growth over the control. Certain of the elements, particularly nickel and cobalt, were highly toxic at more than 4 p. p. m., in the purified solution, and some depression was caused at 4 p. p. m., particularly with nickel.

The use of the factorial design provided a convenient and highly effective method for studying the reaction of the organism to these elements, particularly in evaluating interactions.

In the standard solution, the addition of copper, iron, manganese, and zinc did not produce as good growth as could be obtained on the purified solutions to which these elements were added. Furthermore, in the preliminary studies with unpurified solution, it was believed that the amounts of impurities contained might be a serious factor in evaluating the response of the organism through additions of the elements.

The results of three factorial experiments for determining the effect of the presence or absence of copper, iron, manganese, and zinc in purified solutions are discussed. It was found that copper was somewhat valuable at 2 p. p. m. and unimportant at 5 p. p. m., and that it caused a slight depression at 10 p. p. m. With iron, manganese, and zinc present, very marked increases in growth occurred, much more so than could be accounted for by summation of their effects when used singly, thereby indicating very important interactions. Interaction was greatest for combinations of iron and zinc and manganese and zinc.

In subsequent factorial experiments it developed that the unpurified solution gave results equal or superior to those obtained with the purified solution, provided copper was entirely omitted and iron, manganese, and zinc were employed in about the same ratios. It seemed to matter little whether these rates were 2–2–2 or 5–5–5 p. p. m., although later experiments indicted that the 2–2–2 p. p. m. rate might be considered as optimum for growth of the organism in the unpurified solution. The unpurified solution was extremely sensitive to the addition of copper and was very much more sensitive than the purified solution to increasing concentrations of iron and zinc, particularly the latter.

In further experiments in which iron and zinc were varied at four different levels each, while manganese was held constant at 2 p. p. m., the importance of these relative ratios became further evident and the harmful effects resulting from an unbalanced condition were stressed, particularly in the inhibiting effects of increased concentrations of zinc in relation to the iron concentration. One unexplained anomaly was the depressing effect of iron at 10 p. p. m. in the unpurified solution, an effect which largely disappeared at higher concentrations.

In an attempt to determine whether continued growth on artificial media had resulted in the deterioration of the various isolates of the organism, and whether all isolates would utilize equally well the elements that had been shown to be necessary for optimum growth, three new isolates of *Phymatotrichum omnivorum* were compared with each other and with the standard strain, which had been in culture for over a year. The results showed that these isolates could be distinguished by their rate of growth and by their ability to utilize different combinations of iron, manganese, and zinc.

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THEORETICAL ANALYSIS OF SMOKE-COLUMN VISIBILITY[1]

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INTRODUCTION

As part of a general forest-fire-research program in recent years, considerable inquiry has been made into the visibility of smoke from forest fires in an attempt to answer the questions: How far can a lookout see a smoke? What are the factors upon which this visual range depends? In what way and to what extent do these factors exert their influence? In the answer to these questions are embodied principles upon which a forest-fire-detection system should be based and upon which practical administrative action should be taken in establishing lookouts and in posting supplementary observers in time of emergency.

Byram (*4*) [3] has observed variations in visibility distance with haze of a small smoke against a black background and has reasoned that this visibility distance would be modified by variations in background brightness, position of the sun, illumination, and magnification or increase in size of the smoke body. Buck and Fons (*2*) made a laboratory study of the effect of direction of illumination and of suspensoid concentration upon smoke brightness and Buck (*1*), by analysis of forest-fire discovery-time data, has stressed the importance of the angle between sun, smoke, and observer as influencing visibility. There is need for a more complete theoretical analysis of this problem, starting from defined fundamental properties, with a clear understanding of the assumptions and approximations involved. Such an analysis should serve as a basis for the development of ideas and for the improvement and standardization of techniques.

With this aim in mind this paper was prepared, not in any attempt to solve the whole problem of smoke visibility, but rather to treat of the theoretical aspects and to develop equations for visual smoke range to the point where experimental observations are required to evaluate constants and to determine unknown functions. The equations, if acceptable, should serve as a working hypothesis and guide in the experimentation needed for complete or practical solution of the problem.

GLOSSARY OF SYMBOLS

ϕ = Light flux entering the observer's eye.
$d\phi$ = Flux reaching the eye from an infinitesimal element of surface da.
ϕ_1 = Flux reaching the eye from the haze between the observer and the smoke.
ϕ_2 = Flux reaching the eye from the smoke.

[1] Received for publication August 7, 1940.
[2] Maintained in cooperation with the University of California, Berkeley, Calif.
[3] Numbers in italics refer to Literature Cited, p. 178.

ϕ_3 = Flux reaching the eye from the haze between the smoke and the background.

ϕ_4 = Flux reaching the eye from the background in the direction adjacent to the smoke column.

ϕ_5 = Flux reaching the eye from the background in the direction of the smoke column.

ϕ_6 = Flux reaching the eye from the haze between the observer and the background in the direction adjacent to the smoke column.

ϕ_s = Flux reaching the eye from the direction of the smoke column.

ϕ_b = Flux reaching the eye from the direction of the background adjacent to the smoke column.

a = Area in the field of view perpendicular to the line of sight.

da = An infinitesimal element of the surface a.

A = Apparent reflectance (ϑ) of an element of the background adjacent to the smoke column; also used with subscripts s, h, and ϵ to refer to the sunlight, skylight, and earthlight respectively. Apparent luminous reflectance is the ratio of light reflected to light incident on a perfectly diffusing surface of such reflectance that it has the same brightness, viewed at close range, as the sample surface similarly illuminated and viewed. It is a function of the spectral and angular distribution of the incident light and of the direction of view. If the surface were perfectly diffusing, A might be regarded as the intrinsic brightness of the background and as equal to the diffuse reflection coefficient.

B = Brightness, in general.

B_b = Apparent brightness of the background, viewed at a distance.

B'_b = Apparant brightness of the background, viewed at close range.

B_{sb} = Apparent brightness of the smoke, viewed at a distance against a terrestrial background.

B_{sh} = Apparent brightness of the smoke, viewed at a distance against the horizon sky.

B'_{sb} = Apparent brightness of the smoke, viewed at close range against a terrestrial background.

B'_{sh} = Apparent brightness of the smoke, viewed at close range against the horizon sky.

B_h = Apparent brightness of the horizon sky adjacent to the smoke column or to the background.

l = Distance from the observer to the background.

l' = Distance from the observer to the smoke.

l^* = That particular value of l' at which the smoke can barely be distinguished.

v = The fractional difference between two brightnesses barely distinguishable by the normal human eye.

x = General distance along the eye-to-smoke axis.

Z_1 = The angular scattering function for an average particle of hazy atmosphere. Z_1 is a function of the spacial distribution of the light incident upon the particle as well as of the distribution of the scattered light.

Z_2 = The angular scattering function for an average particle in a volume of smoke. Z_2 is a function both of the spacial distribution of the light incident upon the particle and of the scattered distribution.

$\Delta\omega$ = The solid angle subtended by the pupil of the observer's eye at the element da.

ρ = The coefficient of light attenuation by the hazy atmosphere, defined by $dI = -\rho I dx$ in which dI symbolizes the change in transmitted intensity due to scattering and absorption in an incident beam of intensity I in the distance dx. $(1-\rho)$ = transmission coefficient. ρ = scattering + absorption coefficients. $e^{-\rho x}$ = the fraction of the incident light transmitted through a distance x of hazy atmosphere.

α = The scattering coefficient of the hazy atmosphere, defined by $dI = -\alpha I dx$, in which dI represents the change in transmitted intensity due to scattering alone.

σ = The coefficient of light attenuation by the smoke (analogous to ρ for the haze).

γ = The scattering coefficient of the smoke (analogous to α).

α_1 = The scattering power of a single average particle of the hazy atmosphere.

γ_1 = The scattering power of a single average particle of the volume of smoke.

n = Number of scattering particles.

c = Volume concentration of scattering particles.

θ = The angle of deviation for sunlight of the scattered ray from the path of the incident ray.
ψ = The angle of inclination of the earth-surface element from the horizontal toward the observer.
t = Effective thickness or diameter of the smoke column.
$\bar{d}$ = Mean effective length of the path of the illuminating light from the outer surface into the body of the smoke column.
I = Intensity of incident illumination; also used with subscripts s, h, and e to refer to the sunlight, skylight, and earthlight, respectively.
w = Wind intensity.
m = Magnification.

GENERAL THEORY

A smoke is visible largely because of a contrast in brightness with the adjacent background.[4] This contrast may be expressed in terms of the "contrast ratio" defined as follows:

$$\text{Contrast ratio} = \frac{\text{Apparent brightness of the background}}{\text{Apparent brightness of the smoke column}}$$

Ordinarily, the smoke will appear brighter than its background and the contrast ratio will vary between zero and unity. Zero would mean extreme contrast and maximum visibility. At a ratio of 1, there would be no contrast and the smoke could not be discerned. A sooty smoke or a dense smoke illuminated from behind may appear darker than its background, especially if the latter be the horizon sky, and the contrast ratio as defined would then be greater than unity.

An expression is required for the contrast ratio between a smoke column and its background in terms of defined and measurable quantities.

Consider an eye observing a distant smoke against a solid background as represented in figure 1. The luminous flux to enter the pupil of the eye from the two directions shown will be ϕ_s[5] and ϕ_b. The former will consist of the sum of ϕ_1, ϕ_2, ϕ_3, and ϕ_5. The latter will equal $\phi_4+\phi_6$. Our procedure will be to express each one of the quantities ϕ_1, ϕ_2, ϕ_3, ϕ_4, ϕ_5, and ϕ_6 separately in order to determine ϕ_s and ϕ_b. Then, by changing ϕ, flux, to B, brightness, we will have B_{sb} and B_b, the ratio of which will be the contrast ratio that we seek.

It is assumed that in all cases essentially white light or light of constant hue is involved.

FLUX FROM THE BACKGROUND ϕ_4 AND ϕ_5

Imagine an observer viewing a distant background. His eye will focus upon only one surface element at a time, and no more than the one need be considered, since any other would be brought to a focus on the retina at a different point, and it is assumed that the immediate background is of uniform brightness. Let the projection of the element perpendicular to the line of sight be da and the amount of light flux to reach the eye from the element be $d\phi_4$. Consider the background surface inclined toward the observer at an angle ψ from the horizontal, and assume the normal to the plane of the element to

[4] There is also some contrast in hue, but it is very slight for smokes at such a distance that they are barely distinguishable. Accordingly, in this study hue contrast is considered negligible. Movement plays its part in enabling the observer to conclude that some foreign aspect of the landscape is or is not smoke, but not in rendering that object initially visible.

[5] All terms are defined in the Glossary of Symbols, pp. 161–163.

lie in the plane through the sun and the line of sight. Under these conditions, the point observed would be the high light if the background were glossy; hence a natural background would be of maximum brightness and a smoke viewed against it would be least apparent.

Consider for the moment sun illumination only. The background

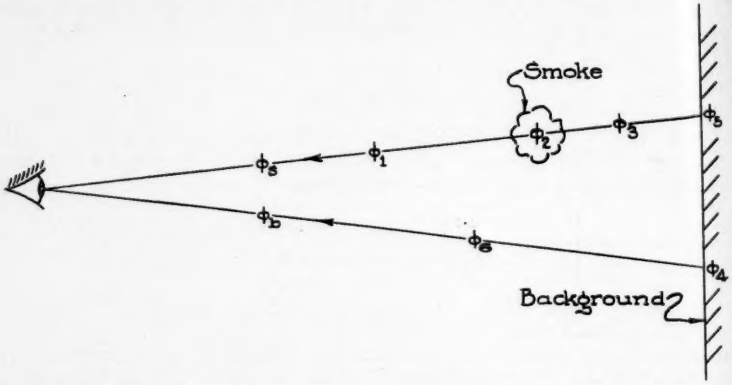

FIGURE 1.—Schematic drawing showing the six sources of the light flux that reaches an observer viewing a body of smoke against a distant background.

will be illuminated by sunlight at an angle θ, as illustrated in figure 2. From the projected element of surface da a certain amount of sunlight will be reflected through the solid angle $\Delta\omega$ and will enter the pupil. If I_s be the full intensity of the sunlight, the illumination in terms of flux per unit area on the inclined surface is $I_s \sin(\theta-\psi)$. The

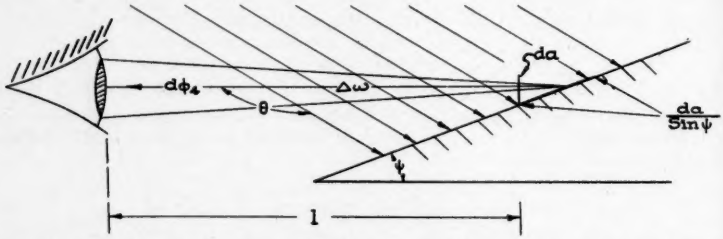

FIGURE 2.—Schematic drawing to show flux reaching the eye of an observer viewing a distant background illuminated by sunlight.

apparent reflected sunlight flux from unit surface is $I_sA_s \sin(\theta-\psi)$, and from the element of surface is $I_sA_s \sin(\theta-\psi)\, da$. $\frac{I_s}{\pi} A_s \sin(\theta-\psi)\, da\, \Delta\omega$ is the portion of this reflected flux directed towards the eye and, since this flux will be diminished by attenuation in transit, the sunlight flux to enter the pupil will be

$$\frac{I_s}{\pi} A_s \sin(\theta-\psi)\, da\, \Delta\omega\, e^{-\rho l}$$

in which $e^{-\rho l}$ is the fraction of the light transmitted through a distance l of hazy atmosphere of attenuation coefficient ρ.

The element of surface is illuminated not only by sunlight but also by skylight and earthlight. These illuminations depend on ψ in an unknown manner, for which the general symbols $f_h(\psi)$ and $f_e(\psi)$ will be used to denote the surface integrals between the normal to the earth-surface element and the horizon over the sky and the earth, respectively. Thus the skylight flux to reach the eye from the surface element will be $I_h A_h f_h(\psi)\ da\ \Delta\omega\ e^{-\rho l}$ and the earthlight flux will be $I_e A_e f_e(\psi)\ da\ \Delta\omega\ e^{-\rho l}$.

The total flux, $d\phi_4$, to reach the eye from the earth-surface element will be the sum of the above three quantities.

$$d\phi_4=\frac{I_s}{\pi} A_s \sin(\theta-\psi)\ da\ \Delta\omega\ e^{-\rho l}+I_h A_h f_h(\psi)\ da\ \Delta\omega\ e^{-\rho l} + I_e A_e f_e(\psi)\ da\ \Delta\omega\ e^{-\rho l} \quad (1)$$

The first (sunlight) term of the right-hand side of equation (1) is ordinarily the predominating one, but it would be absent when the sky is completely overcast or when the background is in shadow. The sunlight term has no real physical significance when $(\theta-\psi)$ is negative. When $\theta=\psi$, there will be grazing incidence and zero illumination by the sunlight. The third (earthlight) term is probably negligible except when the earth is blanketed with snow.

For the sake of simplicity, equation (1) may be put in the form

$$d\phi_4=IA\,f(\theta,\psi)\ da\ \Delta\omega\ e^{-\rho l} \quad (2)$$

in which I is the intensity of illumination from all sources and A is the concomitant apparent luminous reflectance.

Should the light from the background pass through a volume of smoke of attenuation coefficient σ and thickness t, the flux to reach the eye would be $d\phi_5$.

$$d\phi_5=d\phi_4 e^{-\sigma t}=IA\,f(\theta,\psi)\ da\ \Delta\omega\ e^{-\rho l}e^{-\sigma t} \quad (3)$$

For very dark backgrounds and for very dense smokes $d\phi_5$ becomes negligible.

FLUX FROM THE HAZE BETWEEN OBSERVER AND BACKGROUND, ϕ_6

To obtain $d\phi_6$, let da now represent the surface of an infinitesimal cyclinder of haze of length dx (fig. 3). Imagine a myriad of very small particles in this cylindrical element of volume, each scattering independently of the others, so that the scattered intensity is proportional to the number of particles.

The intensity of the light scattered in any particular direction by the infinitesimal volume of hazy air will be proportional to the incident intensity I, to the number dn of scattering centers in the volume and to the ability α_1 of each particle to scatter light. The proportionality constant Z_1 involves the angular distribution of the incident light as well as the angular distribution of the scattered light. α_1 is characteristic of the air molecules and haze particles and represents

a weighted mean for the diversity of kind, size, and shape of scattering particles present. Hence the total light flux scattered by the elemental volume as in figure 3 will be proportional to $I\ dn\ \alpha_1$, or $I\alpha_1\ c\ da\ dx$, or $I\ \alpha\ da\ dx$ in which $\alpha = c\ \alpha_1$. The flux scattered in the direction of the eye will be $Z_1\ I\ \alpha\ da\ dx\ \Delta\omega$ and, of this, $Z_1\ I\ \alpha\ da\ dx\ \Delta\omega\ e^{-\rho x}$ will reach the eye and enter the pupil. This quantity summed up for every similar little element of haze between the observer and the background will be $d\phi_6$.

$$d\phi_6 = Z_1 I\alpha\ da\ \Delta\omega \int_0^l e^{-\rho x} dx = Z_1 I\ da\ \Delta\omega\ \frac{\alpha}{\rho}(1 - e^{-\rho l}) \tag{4}$$

In this integration a uniform haze is assumed to exist between the eye and the background, and the line of sight is assumed to be uniformly illuminated by sunlight, skylight, and earthlight. This latter assumption would be severely violated by an unusually bright or

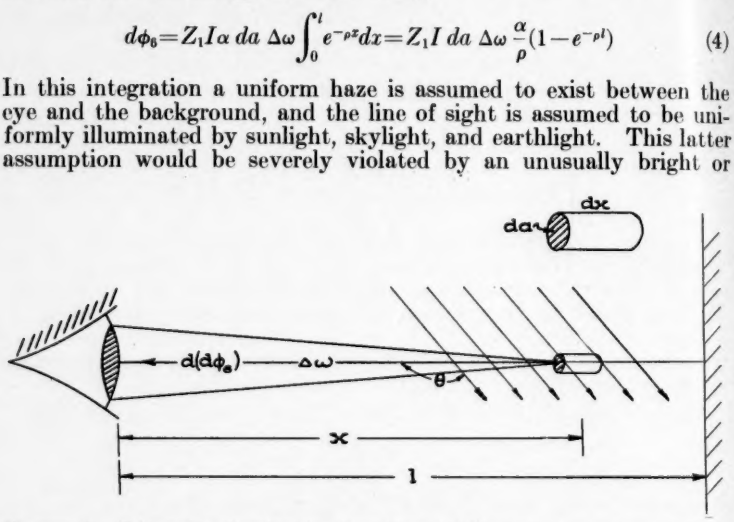

FIGURE 3.—Schematic drawing to show flux reaching the eye of an observer viewing an element of haze illuminated by sunlight.

dark cloud on that part of the horizon which is near the smoke column, or by a shadow cast by a heavy cloud over a part of the line of sight.

FLUX FROM THE HAZE BETWEEN OBSERVER AND SMOKE, ϕ_1

In analogy to equation (4), we may write at once

$$d\phi_1 = Z_1 I\ da\ \Delta\omega\ \frac{\alpha}{\rho}(1 - e^{-\rho l'}) \tag{5}$$

FLUX FROM THE HAZE BETWEEN SMOKE AND BACKGROUND, ϕ_3

In analogy to equation (4) except that in this case the haze light passes through the smoke, which transmits only the fraction $e^{-\sigma t}$, we have

$$d\phi_3 = Z_1 I\ da\ \Delta\omega\ \alpha e^{-\sigma t} \int_{l'}^{l} e^{-\rho x} dx$$

$$d\phi_3 = Z_1 I\ da\ \Delta\omega\ \frac{\alpha}{\rho}(e^{-\rho l'} - e^{-\rho l}) e^{-\sigma t} \tag{6}$$

For dense smoke and smokes close to the background $d\phi_3$ is negligible.

FLUX FROM THE SMOKE COLUMN, ϕ_2

Consider, now, only light scattered from the smoke column. If the angular scattering function for a smoke particle [6] be Z_2 and if I be the incident intensity from all sources, then $Z_2\ I\ dn\ \gamma_1$ is the scattered intensity in a particular direction, such as the direction of the observer, i. e., the flux sent through unit solid angle from dn particles of the matter comprising a volume of the smoke, each on the average with ability to scatter flux denoted by γ_1. Like Z_1, Z_2 is a function of the incident distribution as well as of the sacttered distribution.

Considering the dn particles to be in a cylindrical volume of smoke of da face area and dx length, the intensity of the illumination on this elemental volume will be $I\ e^{-\sigma\bar{d}}$, and the total scattered flux will be proportional to $Ie^{-\sigma\bar{d}}\ \gamma_1\ dn$, or $Ie^{-\sigma\bar{d}}\gamma_1\ c\ da\ dx$, or $I\ e^{-\sigma\bar{d}}\gamma\ da\ dx$ in which c=the concentration of scattering particles in the smoke, and $\gamma=c\gamma_1$.

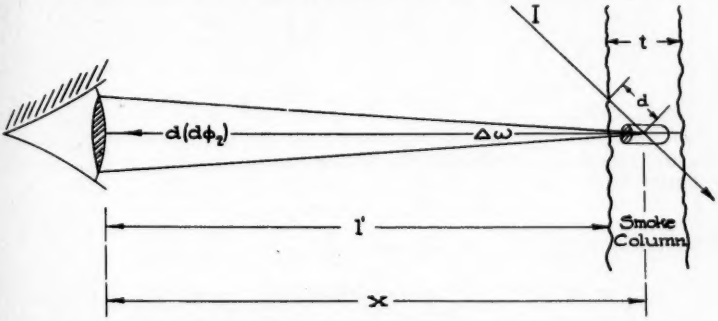

FIGURE 4.—Schematic drawing to show flux reaching the eye of an observer viewing an element of smoke illuminated by sunlight.

The flux scattered in the direction of the eye subtending the angle $\Delta\omega$ will be $Z_2\ I\gamma\ da\ dx\ \Delta\omega e^{-\sigma\bar{d}}$. Of this flux scattered from the elemental volume of smoke toward the eye, the flux to reach the front edge of the smoke column will be $Z_2\ I\ \gamma\ da\ dx\ \Delta\omega\ e^{-\sigma(x-l')}\ e^{-\sigma\bar{d}}$. This is illustrated in figure 4. The flux scattered from the volume $t\ da$ toward the eye is

$$Z_2 I\gamma\ da\ \Delta\omega\ e^{-\sigma\bar{d}} \int_{l'}^{l'+t} e^{-\sigma(x-l')}dx \text{ or } Z_2 I\frac{\gamma}{\sigma}\ da\ \Delta\omega\ (1-e^{-\sigma t})e^{-\sigma\bar{d}} \qquad (7)$$

In this integration it is assumed that γ and σ are not functions of x; that is, that the smoke concentration is uniform in all parts. $\bar{d}$, by definition, is not a function of x.

The flux to reach the eye from the volume $t\ da$ is $d\phi_2$.

$$d\phi_2=Z_2 I\frac{\gamma}{\sigma}\ da\ \Delta\omega\ (1-e^{-\sigma t})e^{-\sigma\bar{d}}e^{-\rho l'} \qquad (8)$$

[6] Since the particles which scatter light are not only the smoke ash suspensoid but also droplets of water vapor and molecules of gas and air, all both charged and uncharged, Z_2 must include the effective scattered light distribution for the mixture as encountered in practice.

APPARENT BRIGHTNESS OF A BACKGROUND

The luminous flux which reaches the observer's eye from the direction of the background is ϕ_b. It comes not only from the background but also from the intervening haze, as shown in figure 1. Whence,

$$d\phi_b = d\phi_4 + d\phi_6$$

By equations (2) and (4)

$$d\phi_b = IAf(\theta,\psi)\, da\, \Delta\omega\, e^{-\rho l} + Z_1 I\, da\, \Delta\omega\, \frac{\alpha}{\rho}(1-e^{-\rho l}) \tag{9}$$

Since brightness is defined as flux per unit solid angle per unit area,

$$B = \frac{d\phi/\Delta\omega}{da} \tag{10}$$

with which equation (9) may be converted to

$$B_b = IAf(\theta,\psi)e^{-\rho l} + Z_1 I\frac{\alpha}{\rho}(1-e^{-\rho l}) \tag{11}$$

APPARENT BRIGHTNESS OF A SMOKE COLUMN

The luminous flux which reaches the observer's eye from the direction of the smoke column is ϕ_s. It arises from four sources as shown in figure 1:

$$d\phi_s = d\phi_1 + d\phi_2 + d\phi_3 + d\phi_5$$

By equations (5), (8), (6), (3), and (10),

$$B_{sb} = Z_1 I\frac{\alpha}{\rho}(1-e^{-\rho\gamma}) + Z_2 I\frac{\gamma}{\sigma}(1-e^{-\sigma t})e^{-\sigma\bar{d}}e^{-\rho\gamma}$$

$$+ Z_1 I\frac{\alpha}{\rho}(e^{-\rho l'} - e^{-\rho\gamma})e^{-\sigma t} + IAf(\theta\psi),e^{-\rho l}e^{-\sigma t} \tag{12}$$

THE CONTRAST RATIO

By definition, contrast ratio $= \frac{B_b}{B_{sb}}$, which by equations (11) and (12) becomes

$$\frac{B_b}{B_{sb}} = \frac{Af(\theta,\psi)e^{-\rho l} + Z_1\frac{\alpha}{\rho}(1-e^{-\rho l})}{Z_1\frac{\alpha}{\rho}(1-e^{-\rho l'}) + Z_2\frac{\gamma}{\sigma}(1-e^{-\sigma t})e^{-\sigma\bar{d}}e^{-\rho l'} + Z_1\frac{\alpha}{\rho}(e^{-\rho l'} - e^{-\rho l})e^{-\sigma t} + Af(\theta,\psi)e^{-\rho l}e^{-\sigma t}} \tag{13}$$

From the standpoint of forest-fire detection the important quantity is l^*, the maximum distance that a smoke of defined characteristics can be seen. The distance of the smoke from the observer l' becomes l^* when the contrast ratio has the value $(1 \pm v)$, in which v is the threshold of brightness contrast. Making this substitution in (13) and arranging explicitly for l^* we have

$$l^* = \frac{1}{\rho} ln \frac{(1-e^{-\sigma t})\left(Z_2\frac{\gamma}{\sigma}e^{-\sigma\bar{d}} - Z_1\frac{\alpha}{\rho}\right)}{\frac{1}{1\pm v}\left[Af(\theta,\psi)e^{-\rho l} + Z_1\frac{\alpha}{\rho}(1-e^{-\rho l})\right] - Z_1\frac{\alpha}{\rho}(1-e^{-\sigma t}e^{-\rho l}) - Af(\theta,\psi)e^{-\rho l}e^{-\sigma t}} \tag{14}$$

Equation (14) symbolizes the maximum distance that a smoke of defined characteristics γ, Σ and t can be seen by an eye of normal vision v, in terms of the haziness of the atmosphere ρ and α, the distance l of the background, the distribution of the incident light and reflection characteristics of the background A, the inclination ψ of the background, the direction θ of the sun's rays, and two quantities, Z_1 and Z_2, which are functions of the distribution of the incident and scattered light, respectively for atmospheric haze and the kind of smoke under observation. The cancellation of I from equation (13) so that it does not appear in (14) indicates that intensity of illumination, within those limits beyond which it may effect v, has no influence upon the visibility of a smoke.

The preceding discourse has been a purely theoretical development of a complete expression for the visibility of a smoke column in terms of fundamental properties of smoke, haze, and background. If the result (equation 14) were simpler, l^*, would be immediately calculated by evaluating all quantities on the right-hand side of the equation. This is not feasible in an expression of such complexity, which, for practical use, must first be simplified. In the following simplification certain reasonable approximations are made, indefinite functions are replaced by quantities amenable to experimental determination, and variables of minor importance are omitted entirely from consideration. Further, in order to allow for vagaries of smoke, it is suggested that certain composite functions of variables be evaluated from observations of l^* made on actual smokes.

A SIMPLIFICATION OF EQUATION (14) [7]

The form of equation (14) can be improved by the use of "close-up" brightness ratios as follows:

ELIMINATION OF Z_1

In figure 3, if the eye be directed towards the horizon sky, the flux to enter the pupil would be expressed by equation (4) with l set at infinity, namely, $Z_1 I\, da\, \Delta\omega \frac{\alpha}{\rho}$. This quantity, divided by $da\, \Delta\omega$, is the horizon brightness, B_h.

$$B_h = Z_1 I \frac{\alpha}{\rho} \tag{15}$$

ELIMINATION OF Z_2, σ, γ, AND t.

The light flux from the direction of the smoke reaching an observer standing close in front of the smoke would be $\frac{d\phi_2 + d\phi_3 + d\phi_5}{e^{-\rho l'}}$. Hence, from equations (8), (6), and (3), B_{sb}, the brightness of a smoke viewed at close range against a terrestrial background, would be

$$B'_{sb} = Z_2 I \frac{\gamma}{\sigma}(1 - e^{-\sigma t})e^{-\sigma \bar{d}} + Z_1 I \frac{\alpha}{\rho} e^{-\sigma t} \frac{(e^{-\rho l'} - e^{-\rho l})}{e^{-\rho l'}} + IAf(\theta,\psi)\frac{e^{-\rho l}}{e^{-\rho l'}} e^{-\sigma t} \tag{16}$$

[7] The writer is indebted to Deane B. Judd of the Colorimetry Section of the Bureau of Standards, Washington, D. C., for suggesting the use of the conception of apparent luminous reflectance and "close-up" brightness ratios employed in the simplification of equation (14).

ELIMINATION OF $Af(\theta,\psi)$

When the background is viewed at close range, B_b becomes B'_b and equation (11), for $l=0$, becomes

$$B'_b = IAf(\theta,\psi) \tag{17}$$

Substituting from (15), (16), and (17) in equation (13) the expression for the contrast ratio becomes

$$\frac{B_b}{B_{sb}} = \frac{B'_b e^{-\rho l} + B_h(1-e^{-\rho l})}{B_h(1-e^{-\rho l'}) + B'_{sb}e^{-\rho l'}}, \tag{18}$$

and equation (14) becomes

$$l^* = \frac{1}{\rho} ln \frac{1-\frac{B'_{sb}}{B_h}}{1-\frac{1}{1\pm v}\left(\frac{B'_b}{B_h}e^{-\rho l}+1-e^{-\rho l}\right)} \tag{19}$$

ELIMINATION OF l

For practical purposes it would seem possible to eliminate one more variable by assuming the background to be always a certain small distance beyond the smoke, as, for instance, 0.1 mile for smokes on the near side of a mountain ridge, and 3 miles for smokes on the crest or remote side of a ridge, values best determined by field study of each locality. This having been done, equation (19) could be written in the form:

$$l^* = \frac{1}{\rho} ln \frac{\left(\frac{B'_b}{B_h}-1\right)e^{-\rho(l-l^*)}+\left(1-\frac{B'_{sb}}{B_h}\right)(1\pm v)}{\pm v} \tag{20}$$

in which l-l^* would be assigned the particular value decided upon.

Equation (20) is a pleasing simplification of equation (14). It implies that when v and l-l^* have been established for actual field conditions, the maximum distance that a given smoke could be seen depends upon three quantities ρ, $\frac{B'_b}{B_h}$, and $\frac{B'_{sb}}{B_h}$, functions of the haze, of the background, of the smoke, and of the manner of illumination. It leaves unsolved the difficult problem of evaluating the close-up ratios in terms of the nature of the background, the haze, the smoke, the illumination, and the sun's azimuth.

VISIBILITY DISTANCE OF SMOKE AGAINST THE HORIZON

In the foregoing, the lookout has been considered to be observing the smoke against a mountainous background. The smoke may of course be viewed against the sky. Any formula derived above can be changed to meet this alteration of conditions by setting $B'_b = B_h$ and $l=\infty$.

B'_{sb} then becomes B'_{sh}, and B_{sb} becomes B_{sh}.

Equation (14) for horizon background becomes

$$l^* = \frac{1}{\rho} ln \frac{(1-e^{-\sigma l})\left(Z_2\frac{\gamma}{\sigma}e^{-\sigma\bar{a}}-Z_1\frac{\alpha}{\rho}\right)}{Z_1\frac{\alpha}{\rho}\frac{\pm v}{1\pm v}} \tag{21}$$

Similarly, equation (20) takes the form

$$l^*=\frac{1}{\rho}ln\frac{(1\pm v)\left(1-\frac{B'_{sh}}{B_h}\right)}{\pm v} \tag{22}$$

In terms of the fundamental quantities, $B'_{sh}=\frac{d\phi_2+d\phi_3}{da\ \Delta\omega}$ with $l'=0$ and $l=\infty$, or

$$B'_{sh}=Z_2I\frac{\gamma}{\sigma}(1-e^{-\sigma t})e^{-\sigma\bar{d}}+Z_1I\frac{\alpha}{\rho}e^{-\sigma t} \tag{23}$$

which shows B'_{sh} to depend on illumination and on characteristics of the smoke and haze.

VISIBILITY AND APPARENT SIZE OF SMOKE COLUMN

No mention has been made of the size of the smoke, although the maximum distance a smoke can be seen depends on its apparent dimensions, particularly the subtended angular width. It would seem possible to allow for size as a formal variable by considering that, whereas v may be constant for neutral objects of large to moderate apparent size, it is not constant for objects of small subtended angular dimensions. Indeed, v is appreciably larger above 2 minutes and must be rather great for objects approaching the minimum angle of visual resolution. Moreover, these small angles may be especially important in forest-fire smoke detection.

Under good laboratory conditions, v is often taken to be 0.02, but actually it is a many-functioned variable dependent upon illumination, glare, adaptation of the eye, sharpness of image, as well as size and shape of object. Consequently, it would seem necessary to determine v from actual smokes under typical field conditions and thus to relate v experimentally to apparent size. In this way v would be expressed as a function of the subtended width, t/l^*, perhaps as an empirical equation of the form

$$v=p(t/l^*)^{-q}+r \tag{24}^{8}$$

After evaluation, equation (24) may be utilized to replace v in (14) and (20) with its equivalent function in terms of t and l^*. The resulting equations would involve l^* as the only unknown and would be solutions of the problem for both size of smoke and brightness of smoke.

A telescope has the effect of increasing apparent dimensions without altering relative brightnesses. It might be argued that binoculars could be used to increase the subtended angular width to $m\frac{t}{l^*}$ (in which m is the magnification) and to extend correspondingly the

[8] The constants p, q, and r in equation (24) may be evaluated approximately to give

$$v=23\cdot10^{-5}(t/l^*)^{-1.73}+0.03$$

from the data of Helmholtz as presented by Middleton (*10, p. 32*). This equation may be substantially correct for subtended angles down to one-fourth minute. Nearly the same equation holds for Byram's observations (*4*) on smoke, v being obtained by applying equation (14) to his data and conditions, on the assumption that the diameter of the smoke body at the time of observation varied regularly with distance from 8 to 12 feet. Assuming a constant diameter of 10 feet, Byram's data lead to the relation,

$$v=0.021+1.18\cdot10^{-5}(l^*t).$$

radius of vision. In practice, however, binoculars are rarely used to detect forest-fire smokes. Rather are they used to identify a smoke as such after it has been discovered. A magnification of m times is not the equivalent of an $m$fold increase in smoke diameter. The former increases visibility through increase in subtended angle, the latter not only through increase in subtended angle but also through increase in brightness because of greater smoke depth along the line of sight.

SMOKE VISIBILITY AND WIND

Visibility of the smoke from an incipient forest fire is influenced to an important extent by wind intensity and turbulence, velocity gradient, and wind direction relative to the observer-smoke axis. This influence is quite complex and is exerted through increase in the rate of burning, control of the completeness of combustion and of the amount and quality of suspensoid, dispersion of the suspensoid and lowering of its concentration, change in the size and volume of the smoke, and alteration of the geometrical shape and the depth through which the observer sights. The effect of wind is thus upon size t, optical concentration σ, and quality $\frac{\gamma}{\sigma}$. Since l^* has been expressed in terms of these quantities, wind as a separate variable has not entered into the theory. Nevertheless, in practical experimentation upon smoke visibility, in view of the impossibility of producing a smoke column always of given diameter, concentration, and quality and of the difficulty of measuring these quantities, it may prove expedient to evolve suspensoid at a constant rate by artificial control and then to ascertain the effect of wind on the visibility of the smoke thus produced. This procedure will be discussed below under proposal III for the estimation of smoke-visibility distance.

THE "STANDARD SMALL SMOKE"

To the question, "How far can a smoke be seen?" no answer can be given without first asking, "What kind of a smoke?" Throughout the above discussion we have constantly employed such phrases as "a given smoke" or "a smoke of defined characteristics." By given or defined is meant that the values for γ, σ, and t of the smoke are known or that the quality, concentration, and size have been otherwise established.

It has been customary in experimental work on smoke to employ uniformly-formulated smoke compounds in the form of candles or pots to evolve a smoke or fume which is referred to as the "standard small smoke." Although a single arbitrary smoke can fulfill only one set of γ, σ, and t values, and the permissibility of taking this "standard smoke" as a unit of measurement is questionable, it is both simple and convenient to express visibility as the distance at which such a standard small smoke can be seen.

Although the standard small smoke must be chosen arbitrarily, to be serviceable it should be made to duplicate in appearance typical smokes as they are first detected in fire-protection practice. This duplication must be in respect to all the properties of smoke that enter into equation (14), namely size, t, optical concentration, σ,

quality (largely sootiness), $\frac{\gamma}{\sigma}$, and angular scattering function, Z_2. If the standard smoke chosen be too small, its visibility may be determined almost wholly by subtended angular width; such factors as position of the sun and optical density of the haze, which ordinarily are very important, may be insignificant. In other words, the chosen smoke must be typical in size and concentration; it must not be whiter or darker, heavier or lighter, than the just-detected smokes from the forest fuel of chief interest; and it must look like the latter from all directions. If these conditions are met the calculated visual distances will be of the same order of magnitude as distances encountered in practice.

ESTIMATION OF SMOKE VISIBILITY DISTANCE

For the practical utilization of this theory of smoke visibility, several procedures suggest themselves. In equation (14), 12 independent variables compose the maximum visibility distance, l^*. Limiting consideration to a standardized smoke reduces these 12 to 9. Not all 9 are of equal importance and some may be neglected, taken as constant, or expressed only qualitatively without greatly increasing the standard error in the estimation of l^*. It would seem possible to avoid the complication of 2 of these 9 (l and v) by the following expedients.

Equation (14) may be thrown into the following form:

$$l^*=\frac{1}{\rho}ln\left[e^{-\rho(l-l^*)}\left(\frac{Af(\theta,\psi)-1}{Z_1\alpha/\rho}\right)\left(\frac{1-e^{-\sigma l}(1\pm v)}{\pm v}\right)+\left(1-\frac{Z_2}{Z_1}\frac{\gamma}{\sigma}\frac{\rho}{\alpha}(1-e^{-\sigma l})e^{-\sigma d}-e^{-\sigma l}\right)\frac{1\pm v}{\pm v}\right] \tag{25}$$

in which l is introduced as the difference $(l\text{-}l^*)$, which better elucidates its physical role. Between the smoke column and the background there exists illuminated haze scattering light to the eye of the observer, imposing added brightness upon the background, and diminishing the contrast ratio. In forest-fire-detection systems, lookouts are so stationed that the major portion of the forest area is in direct view. Accordingly, smokes are usually seen with rising ground or ridges as backgrounds. Under these circumstances $(l\text{-}l^*)$ would be small, possibly negligible, and could often be taken as constant without much error.

Instead of measuring every independent factor and then calculating the dependent variable (l^*), it is desirable to observe the latter directly, in order empirically to evaluate functions and quantities that do not easily lend themselves to direct measurement. To observe l^* in this way has the advantage of automatically including the function of the vision v and introducing its effect into all empirical relationships so determined. This is the method advocated in all but the final proposal listed below.

In these proposals are successively included the more important variables, leading progressively to increasing complexity but to diminishing standard error in the estimation of l^*. That proposal should be chosen which fulfills the required accuracy without too great complication.

I. Equation (14) might be reduced in form to $l^*=\frac{P}{\rho}$ in which P is the complex logarithm which, for the purpose of extreme simplification, would be taken as a constant and evaluated by observing the distance (l^*) that the standard small smoke could be seen through a hazy atmosphere of attenuation coefficient ρ. In view of the dependence of the more distant observations upon v, as explained above, it would be more rational to plot the data in the form l^* vs. $\frac{1}{\rho}$ and thus to allow for the possibility of finding ρl^* somewhat less at high values of l^* than at low values.

This plot might be made for two conditions, smoke in sunlight and smoke in shade.

The coefficient, ρ, can be evaluated by a hazemeter (*3*) which on the basis of the Koschmieder (*6*) (*7*) equation,

$$\frac{B_b}{B_h}=1-e^{-\rho l},$$

can be used to measure the ratio of the horizon brightness to the brightness of a black target, l miles away; or, following the suggestion and equation of Löhle (*8*),

$$\frac{B_1}{B_2}=\frac{1-e^{-\rho l_1}}{1-e^{-\rho l_2}}$$

the brightness ratio of two black targets at different distances, l_1 and l_2 from the observer. Both equations assume uniform haze and uniform illumination over the line of sight.

II. Equation (14) might be rewritten as $l^*=\frac{Q}{\rho}$ in which Q is assumed to be a function of θ only. θ is the supplement of the angle between observer, smoke, and sun, the smoke being at the vertex. Since the sun's rays are parallel, θ is also the angle between sun, observer, and smoke, with the observer at the vertex. Of the several variables on which Q actually depends, θ is chosen as one of primary importance. From this suggestion, l^*, ρ, and θ would be determined for the standard small smoke. From these data Q ($=\rho l^*$) would be plotted in graphic relation to θ, or preferably, because of the possible effect of v, l^* against θ with ρ as a third variable. Thereafter, with θ and ρ known, l^* for the standard small smoke could be estimated from this plot to the degree of accuracy allowed by the above assumption. This plot might be made for smoke in shade as well as for smoke in sunlight.

III. It is probable that the standard error of proposal II would still be too high for reasonable satisfaction and that the independent variables of next greatest importance should be included. These may be t, σ, and $\frac{\gamma_1}{\sigma}$ which, despite the fact that a standard smoke is being considered, are not constant but are influenced by wind which acts directly on size, shape, and density of the smoke column.[9] In view

[9] Air temperature and relative humidity have effects on smoke, but for atmospheres of the forest-fire season well above the dew point, they may not be of major importance.

of the influence of the wind, equation (14) could take the form $l^* = \frac{R}{\rho}$, in which R would be considered a function not only of θ but also of w, the wind intensity. In this case, experimental data on the standard small smoke would be gathered for l^*, ρ, θ, and w and a plot made of R $(=\rho l^*)$ against θ for various wind intensities, as illustrated in figure 5 (in which the curves are drawn "wavy" to indicate that their true forms are as yet unknown).

As mentioned in the discussion of smoke visibility and wind, the direction of the wind relative to the observer's line of sight is important. A smoke being blown along this axis would undoubtedly be more clearly visible than the same smoke moving across the axis. Limitation of experimental observations to smoke in the cross wind, as far as practicable, would make the estimated value of l^* tend toward a minimum. The alternative would be to determine the data of figure 5 for both cross and longitudinal winds and possibly for intermediate directions.

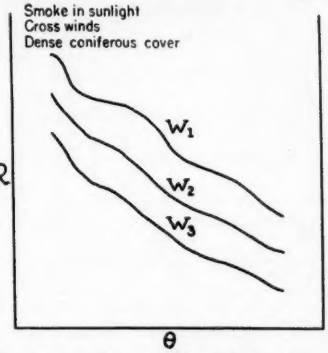

FIGURE 5.—Illustration of the way the relation between R, θ, and w may be shown graphically for particular types of illumination, wind direction, and background.

Furthermore, since the vertical distribution of wind is different for different forest types (open grassland, brush, and woodlands) (*5*) and since the diffusion of smoke as it rises through the forest canopy is influenced by this vertical distribution, the cover type in which visibility observations are made should be specified.

Since ρl^* may vary with v when all other factors are held constant, preferable to figure 5 would be a three-dimensional figure with l^* plotted on one axis.

IV. Of the variables affecting vsiibility distance, procedure III includes only three, and leaves it understood that all variations in background, illumination of background, illumination of smoke and haze, and quality of haze, are relatively immaterial. Of these, the one that should next be considered is probably background, symbolized by A in equation (14). For practical purposes descriptive terms might be employed such as ponderosa pine woods, yellow grass, gray snag field, brown talus rock, etc. Accordingly, to allow for background differences, the graph shown in III would be considered as limited to one particular type of background.

V. The angle of inclination ψ, occurring as one of the factors in equation (14), might be expressed in terms of degrees or percent slope. Practically, however, ψ is of minor importance as long as the earth element is not inclined so far as to approach grazing incidence or to be in its own shadow. A perfectly diffusing surface appears of the same brightness at all inclinations, and to the extent that pine woods, for instance, approach perfect diffusion the less influence has ψ. Reflection from a pine-covered mountain comes much nearer to being completely diffuse than unidirectional, and it is probable that ordi-

narily the only consideration that need be given to ψ is to state "Background in sunlight" or "Background in shadow." It is suggested in this procedure that the data for figure 5 be determined for each of these two conditions.

VI. Illumination affects smoke visibility through the quantities A, Z_1, and Z_2. By "illumination" is meant such natural conditions as (1) bright sunlight in a clear sky, (2) a sky veiled with clouds or mist (light, medium, heavy) through which the direct rays of the sun penetrate only in part, (3) a thickly overcast sky which conceals utterly the position of the sun. In practice it would probably suffice to express illumination in qualitative terms such as the above. To what extent variations in illumination play a part in visibility is uncertain, but if extreme variations are encountered illumination must be considered and figure 5 redetermined for an adequate number of gradations.

VII. To the extent that $(l-l^*)$ is negligible, the ρ in the quantity $e^{-\rho(l-l^*)}$ is also negligible, and haze enters into the logarithm of equation (25) only in the form $\frac{\alpha}{\rho}$. In haze of a given quality, the ratio of light absorbed to light scattered (β/α) is a constant, and since $\rho=\alpha+\beta$, $\frac{\alpha}{\rho}$ also is constant. $\frac{\alpha}{\rho}$ might be measured and expressed numerically but it would be easier and probably sufficient simply to describe the quality of the haze as "vaporous," "smoky," "dusty," "industrial," or "misty." If the effects of variation in $\frac{\alpha}{\rho}$ are found to be large, figure 5 should be drawn for each important class of haze.

VIII. Proposal III, with the additions suggested in IV, V, VI, and VII, takes into account all independent variables of equation (14) to provide a complete solution. Equation (20) offers another way to include all variables in estimating visibility distance.

The two close-up brightness ratios in equation (20) are quantities which could be measured experimentally against a nearby black background. According to equations (15), (16), and (17), they are in fundamental terms as follows:

$$\frac{B'_b}{B_h}=\frac{Af(\theta,\psi)}{Z_1\alpha/\rho} \tag{26}$$

$$\frac{B'_{sb}}{B_h}=\frac{Z_2}{Z_1}\frac{\gamma}{\sigma}\frac{\rho}{\alpha}(1-e^{-\sigma t})e^{-\sigma\bar{d}} \tag{27}$$

From these relations, it is evident that for a specified type of illumination (A, Z_1, Z_2) for a given background (A, ψ) and for a smoke of defined characteristics (γ, σ, t), the ratios will vary with the sun's azimuth (θ) and the quality of the haze $\left(\frac{\alpha}{\rho}\right)$. For a standard small smoke, γ, σ, and t will be constant except as they are affected by winds. On this basis it would be possible to prepare graphs of the kind illustrated in figure 6. By measuring w and θ and converting them into their functions by use of the plots of figure 6, by determining ρ, by

knowing the value of v, and by assuming a reasonable value for $(l-l^*)$, the observer can determine every quantity in equation (20) except l^*, which is immediately calculable.

This procedure has the advantage of providing a direct test of the validity of the theory by comparison of observed and calculated values of l^*. Its disadvantages lie in the necessity of establishing the relation between v and t/l^* and of predetermining the close-up brightness ratios on that part of the smoke column which is most important in rendering the smoke initially visible.

DISCOVERY TIME

In an actual forest fire the perimeter is progressively increasing. As a consequence, t (and possibly σ) varies with time as well as with all the factors which control the rate of spread of the fire. If t in equation (14) were replaced with its equivalent function in terms of

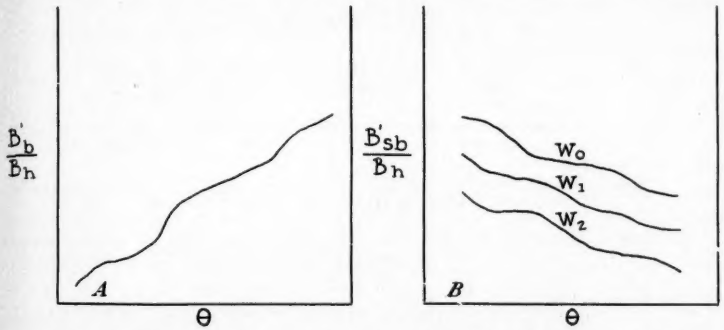

FIGURE 6.—Illustration of the way in which the relation between the close-up brightness ratios and θ may be shown graphically for particular conditions of background, illumination, haze, wind, and smoke: *A*, Clear sky, ponderosa pine forest, background in sunlight, smoky haze; *B*, clear sky, cross wind, ponderosa pine forest, standard smoke candle, smoky haze.

time and rate of spread, the result would be an expression connecting distance between lookout and smoke with the minimum time of discovery. Discovery time is thus indicated to be dependent upon l^*, ρ, θ, v, l, the background brightness, the illumination of the background, the illumination of the smoke, the quality of the haze, the wind direction, the cover type, and the rate of spread of the fire. The time required for a lookout to discover a smoke of rapidly increasing proportions is probably of more practical utility than the radius of vision for a smoke of constant size and density.

SUMMARY

A theoretical analysis has been given of the visibility of a smoke column from a forest lookout for the purpose of clarifying ideas on the physical basis of smoke detection. The visibility of the smoke is considered to be its contrast in brightness with the background. The contrast ratio is expressed in terms of the light flux entering the observer's eye from the smoke, the haze, and the background and the

flux in terms of the scattering and reflecting properties of the various elements. From this expression an equation is derived for the maximum distance a given smoke could be seen under specified conditions. This equation is simplified by substituting "close-up" brightness ratios for elementary scattering functions and by drawing approximations through the introduction of simple assumptions. The equations appertain to various types of illumination and to smokes seen against both sky and terrestrial background.

The theory indicates that the maximum distance at which a smoke is visible is inversely proportional to the amount of haze in the atmosphere, the proportionality factor being the logarithm of a complex quantity involving the sun's azimuth along with certain characteristics of background, the smoke, the illumination, and the vision.

For the practical utilization of the derived equations there are suggested several procedures of increasing complexity leading to diminishing standard error in the estimation of maximum visibility distances.

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COMPARISON OF RATES OF APPARENT PHOTOSYNTHESIS AND RESPIRATION OF DISEASED AND HEALTHY BEAN LEAFLETS [1]

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INTRODUCTION

When a leaf is attacked by a pathogen, it is reasonable to assume that some, if not all, of its physiological processes are affected. Increased respiration and transpiration, delayed translocation, increased or decreased photosynthesis, and an interference with the movement of water and solutes in the vascular system are some of the physiological changes recorded for diseased leaves.

The data presented here consist of comparative measurements of apparent photosynthesis and of respiration on healthy and diseased bean leaflets, respectively. Wherever possible, correlations are indicated between outward symptoms of disease and abnormal physiology.

REVIEW OF LITERATURE

Reed and Crabill (*8*)[3] showed that the presence of aecia of *Gymnosporangium juniperi-virginianae* Schw. reduced the carbon dioxide assimilation of apple leaves 56 to 65 percent. Diseased leaves showed more rapid and also greater total respiration than healthy leaves.

Long (*7*) demonstrated that members of the genera *Puccinia* and *Uromyces* reduce the assimilation rate of oat and wheat leaves by approximately 30 percent. Bailey and Gurjar (*1*) found that wheat plants infected with *P. graminis tritici* Erikss. and Henn. exhibit a reduced rate of respiration.

Yarwood (*13*) reported increased respiration of excised clover leaflets infected by *Erysiphe graminis* DC. or by *Uromyces fallens* Kern.

Kuprewicz (*6*) lists as changes which occur within plants attacked by microorganisms: Decrease in the chlorophyll content and carbon assimilation, increased or decreased respiration, retarded translocation, and increased transpiration.

Smith (*9*) reviews the literature on the metabolism of virus-diseased plants. Mosaic diseases are accompanied by an increase in the total nitrogen and a decrease in the total carbohydrates; yellows diseases produce the opposite effect. Diseased plants often show a delayed translocation. Respiration rates of leaves are higher than normal with young leaves, and lower than normal with old leaves.

[1] Received for publication March 5, 1940. This paper is an abridgment of a thesis presented to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The writer is indebted to Dr. L. M. Massey, under whose guidance the work was undertaken, for his continued interest and help throughout the investigation. The many timely suggestions of Dr. A. J. Heinicke are gratefully acknowledged.

[3] Italic numbers in parentheses refer to Literature Cited, p. 191.

MATERIAL AND METHODS

The writer sought a plant, susceptible to plant pathogens and adaptable to greenhouse cultivation, which produces its leaves in opposite pairs on a common stem. The bean plant (*Phaseolus vulgaris* L.) fulfills these requirements, and a pure line of the variety Red Kidney was selected for study.

The first leaves of the bean plant are simple; the second and succeeding leaves are compound and consist of three leaflets, one terminal and a pair of opposite leaflets. In area, opposite leaflets are very similar, and since they possess a common petiole, their nutrition should also be very similar. It was therefore decided to study pairs of opposite leaflets, comparing the physiology of one member of a pair with that of the other. The following comparisons are presented here: (1) Between the physiology of the two members of a pair of healthy opposite leaflets; (2) between the physiology of a pair of opposite leaflets, one member diseased, the other healthy; and (3), between the physiology of the two members of a pair of diseased opposite leaflets.

Apparent photosynthesis was measured as the amount of carbon dioxide utilized by the bean leaflets, and respiration as the amount of carbon dioxide given off by the leaflets. Air of known carbon dioxide content was passed over the leaflets enclosed singly in envelopes made from heavyweight moisture-proof cellophane, with the edges sealed with Du Pont moisture-proof adhesive No. 395. The air was then bubbled through absorption towers filled with potassium hydroxide. The apparatus used was similar to that developed by Heinicke and Hoffman (*4*) in their studies on the rate of photosynthesis of apple leaves under natural conditions. The difference between the carbon dioxide content of the stream of air passed over the plant material and a comparable stream of normal air determined the photosynthesis of respiration.

Special leaf holders were devised which combined the features of support and air intake. They consisted of two pieces of glass tubing, bent and pushed through a rubber stopper, and held in position by a burette clamp and ring stand. A pair of leaf holders and a pair of opposite bean leaflets enclosed in cellophane envelopes are shown in figure 1.

Bean plants were grown in the greenhouse until the first compound leaf was approximately half its mature size. The plants were then transferred to a darkened room with illumination provided by a pair of 1,000-watt bulbs complete with reflectors, supplying 800 foot-candles at leaf level, 20 inches distant from the source of light. The length of illumination was arbitrarily fixed at 13 hours, from 4:00 a. m. to 5:00 p. m. Plants were kept in this room for 48 hours before physiological measurements were started, and continuously thereafter until studies were terminated.

While the room temperature was fairly constant (72° to 75° F.), that of the air at leaflet level varied from 83° to 88° F. during hours of illumination, occasionally reaching 90°. It is of interest to note that Yoshii (*14*) records greatest assimilation by *Phaseolus* spp. in strong light at 96.8°; decreasing the light intensity reduced the optimum temperature to 80.6°. No measurements were taken of air temperatures within cellophane containers in the present investigation.

Air was supplied to the leaflets at the rate of 2 to 2.5 liters per square centimeter of leaflet surface per hour, as recommended by Heinicke and Hoffman (*4*). The volume of air passed over the plant material was calculated from three readings taken with an air-flowmeter during each 4-hour determination. The carbon dioxide content of the air fluctuated from day to day and, occasionally, during a single day, varying from 0.72 to 0.85 mg. per liter during these studies. The plants were grown in greenhouse soil and watered daily with distilled water.

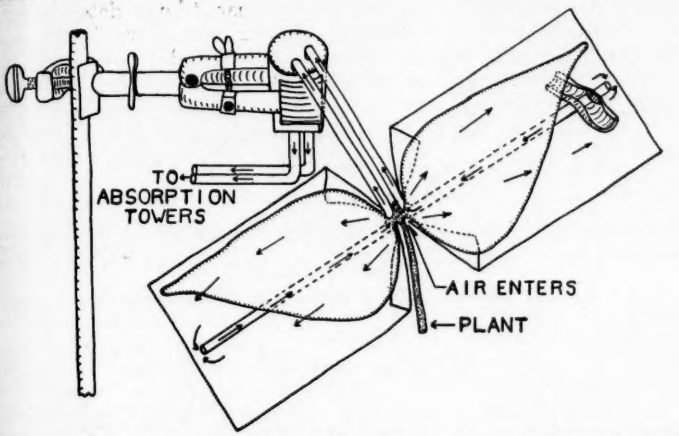

FIGURE 1.—Pair of leaf holders in position, with a pair of opposite bean leaflets enclosed in cellophane envelopes. The path of the air stream is indicated. The third leaflet of the compound leaf of the bean plant is not shown.

A number of investigators have reported the effect of maturity on the physiology of plant tissue. Spoehr (*11*) cites the investigations of Willstätter and Stoll, who showed that photosynthesis was quite high in leaves that were just unfolding, the rate becoming constant after about 9 days of growth. With further aging the rate decreased. Kidd et al. (*5*) showed that respiration of *Helianthus annuus* decreased with maturity. Hée (*3*) found a decrease in respiration with increase in age. Stiles (*12*), discussing the effect of aging on the activity of a leaf, says that "as a leaf gets older, the chlorophyll content increases and along with this the rate of photosynthesis, but not at the same rate. Hence with increase in chlorophyll content the assimilation number falls." To reduce the variable of leaflet age, the first physiological measurements were begun 13 to 15 days after the seed was planted, and data were taken during successive 5-day periods. Data taken on the first pair of leaflets on a plant during the first 5 days were designated "period A" data, data during the following 5 days were designated "period B" data, data taken during the next 5 days were designated "period C" data, and so on. Data on other leaflets on the same plant were similarly arranged; for example, period A for the second pair of leaflets was period B for the first pair, and period A for the third pair was period C for the first pair. By the use of this scheme, it was possible to group together data obtained from leaflets of comparable physiological maturity.

One or two determinations of 4 hours each on apparent photosynthesis were made daily, between the hours of 8:00 a. m. and 12:30 p. m., and between the hours of 12:30 p. m. and 5:00 p. m. One 4-hour measurement of respiration was made daily between 7:00 p. m. and midnight with the plants in the dark. Measurements on apparent photosynthesis taken between 8:00 a. m. and 12:30 p. m. are designated "a. m." data in all tables; those obtained between 12:30 p. m. and 5:00 p. m. are designated "p. m." data.

Data were calculated as milligrams of carbon dioxide assimilated or respired per 100 cm.^{2} of leaflet surface per 4-hour determination. Leaflet areas were obtained from planimeter measurements of contact photographic prints, taken of each leaflet at 3-day intervals. The method of analysis of variance (*10*) was used in the biometrical analysis of all data.

EXPERIMENTAL RESULTS

PHYSIOLOGY OF PAIRS OF HEALTHY LEAFLETS

Apparent Photosynthesis

Seventeen pairs of leaflets, on 10 different bean plants, were studied: 3 pairs on each of 3 plants, 2 on 1, and 1 pair on each of 6 others.

TABLE 1.—*Mean assimilation rates of pairs of healthy, opposite bean leaflets at three periods of maturity, expressed as milligrams of carbon dioxide per 100 cm.^{2} of leaflet surface per 4-hour determination*

[Each mean is the average of 8 to 10 individual observations]

Pair of leaflets No.	Leaflet No.	Mean ± S. E. assimilation rates of leaflets at indicated period of maturity [1]		
		Period A	Period B	Period C
		Milligrams	*Milligrams*	*Milligrams*
1	7		48.3±1.9	
	17		45.7±1.9	
2	11		27.0±2.3	
	21		29.1±2.3	
3	32		39.7±3.3	
	42		37.7±3.3	
4	37		24.7±3.1	
	47		26.9±3.1	
5	77	46.7±3.3		50.0±3.0
	87	39.0±3.3		46.5±3.0
6	110	22.6±4.6		
	210	26.2±4.6		
7	111	35.7±4.4		
	211	36.3±4.4		
8	312	35.8±4.9		
	412	30.1±4.9		
9	313	24.7±4.9		
	413	25.6±4.9		
10	314	32.8±4.4		52.3±5.6
	414	37.7±4.4		51.6±5.6
11	713		44.8±3.1	
	813		43.5±3.1	
12	714	23.6±4.6		
	814	27.3±4.6		
13	715	34.1±4.4		37.6±4.4
	815	34.6±4.4		33.6±4.4
14	716	39.5±5.6		44.7±9.7
	816	42.5±5.6		46.0±9.7
15	913	29.6±4.1		
	1013	28.3±4.1		
16	914	36.1±4.4		46.3±4.4
	1014	28.6±4.4		38.1±4.4
17	915	48.5±4.6		52.7±4.4
	1015	56.7±4.6		55.6±4.4
Average		34.0±1.7	37.1±2.9	45.9±2.1

[1] Description in text, p. 181.

A few pairs were members of the second, third, and occasionally the fourth compound leaf, but the majority of the pairs studied were those of the first compound leaf. Data on apparent photosynthesis are presented in table 1, with each figure the mean of 8 to 10 individual 4-hour observations.

The results given in table 1 show differences as high as, or higher than, 15 percent between the assimilation rates of the members of a pair of healthy, opposite leaflets. These differences are not statistically significant. Some of the differences between assimilation rates of leaves of comparable physiological maturity (periods A, B, or C) are significant.

In the present studies, the effect of maturity of a bean leaflet was marked. The leaflets were studied for approximately 15 days, and the assimilation rate was highest during the last 5-day interval (period C), even though the data were calculated on a unit-area basis. It is not known whether the maximum rate of assimilation was reached; with further increase in age, the rate should decrease steadily until abscission occurs.

No data were obtained on the respiration rates of pairs of opposite healthy leaflets.

PHYSIOLOGY OF PAIRS OF LEAFLETS

One Leaflet Healthy, The Other Diseased

Thirteen pairs of leaflets, one member healthy and the other diseased, were studied. Anthracnose, a disease easily reproduced under controlled conditions, caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav., was selected, and the gamma strain (*2*) used throughout the investigations. The fungus produces necrotic spots on both surfaces of the bean leaflet, but more predominantly on the lower surface. The veinal system is invariably attacked, though interveinal lesions are not uncommon. Secondary symptoms include loss of chlorophyll, desiccation of a part or all of a leaf, and sometimes death, especially when the leaflet is infected in a juvenile stage.

Preliminary studies failed to show a correlation between the amount of inoculum and the severity of subsequently developed symptoms. A spore suspension, which appeared slightly cloudy when held to the light, was applied to both surfaces of the leaflets with a camel's-hair brush. One member of each pair of leaflets was inoculated, the other member similarly treated except that only sterilized water was used. Inoculated plants were well watered, placed for 36 to 48 hours in a moist chamber at approximately 75° F., and then transferred to the illumination chamber. Symptoms usually appeared within 24 to 72 hours after inoculation and measurements of apparent photosynthesis or respiration or both were started immediately following transfer to the chamber, or as soon thereafter as convenient.

Growth Rates

Bean leaflets affected with anthracnose were occasionally visibly smaller than healthy companion leaflets. Measurements on area were taken at 3-day intervals on 41 pairs of healthy opposite leaflets, and on 21 pairs of opposite leaflets with one member healthy and the other diseased, to determine whether retardation in growth was a

constant symptom following infection with *Colletotrichum lindemuthianum*. Inoculation with the fungus was performed immediately following the initial measurements. Average findings are presented in table 2.

TABLE 2.—*Mean percentage increase in the area of members of pairs of healthy opposite leaflets and of members of pairs of opposite leaflets with one member healthy and the other infected with Colletotrichum lindemuthianum*

[Results from 41 pairs of healthy opposite leaflets and 21 pairs of opposite leaflets with 1 member healthy and 1 diseased]

Condition of members of pairs of opposite leaflets	Approximate time between initial and final measurements	Mean increase in area±S. E.	Difference ±S. E.
	Days	*Percent*	*Percent*
Healthy	25	81.84±1.82	
Do	25	77.85±1.82	3.99±2.57
Do	12	18.96± .97	
Diseased	12	[1] 13.40± .97	5.56±1.37

[1] Leaflets inoculated when past juvenile stage, hence the percentage increases in area are lower than those given for pairs of healthy leaflets.

The results showed that between the members of pairs of healthy opposite leaflets there was no significant difference in percentage increase in area (3.99±2.57), but between the members of pairs of opposite leaflets with one member healthy and one diseased there was a significant difference in percentage increase in area (5.56±1.37). The difference between these two figures, approximately 1.6 percent, represents the growth restriction due to infection by *Colletotrichum lindemuthianum*.

APPARENT PHOTOSYNTHESIS

Data obtained on apparent photosynthesis of the pairs of opposite leaflets studied, the approximate time elapsing between planting of seed and inoculation of leaflet and between inoculation and first measurement of apparent photosynthesis, are given in table 3. The leaflets were under observation for varying lengths of time depending on symptom development and intensity, and on convenience of observation. A single 4-hour reading per day, between the approximate hours of 8:00 a. m. and 12:30 p. m., was taken on the majority of the pairs of leaflets. Data are given as mean assimilation rates, each mean representing the average of three to five individual 4-hour readings. The relation of age to the assimilation rate is indicated by separation of data into periods as was done in table 1.

The figures for mean assimilation when all data are combined are as follows: For healthy leaflets, 26.29±1.04; for diseased leaflets, 19.99 ±1.04. The difference is 6.30±1.48.

The majority of the inoculated leaflets showed a reduced rate of assimilation as compared with healthy companion leaflets, but only two leaflets, 36 and 636, showed individual reductions large enough to be within the range of significance. However, when the mean assimilation rate of diseased leaflets is compared with the mean assimilation rate of healthy leaflets for all of the pairs, a 24-percent reduc-

TABLE 3.—*Mean assimilation rates of pairs of opposite bean leaflets at five periods of maturity, when one member was healthy and the other was infected with Colletotrichum lindemuthianum, expressed as milligrams of carbon dioxide per 100 cm.² of leaflet surface per 4-hour determination*

[Each mean for the different periods of maturity is the average of three to five observations]

Pair of leaflets No.	Leaflet No. and condition	Time between sowing of seed and inoculation	Time between inoculation and first measurement	Mean assimilation rates in morning and afternoon of leaflets at indicated period of maturity [1]							Mean of all observations ±S. E.	Difference± S. E.
				Period A		Period B		Period C	Period D	Period E		
				A. m.[2]	P. m.[3]	A. m.[2]	P. m.[3]	A. m.[2]	A. m.[2]	A. m.[2]		
		Days	*Days*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
1	14, diseased	14	3	20.1 (1)	15.3 (1)	24.9 (1)	1.8 (1)				19.2±3.4	5.2±4.8
	24, healthy			27.0	26.1	22.3	20.1				24.4±3.4	
2	36, diseased	14	3	36.7 (1)	37.8 (1)	25.5 (2)	8.7 (2)	29.9 (3)	35.6 (3)	36.3 (3)	32.4±2.9	10.2±4.2
	46, healthy			42.8	49.0	39.9	12.0	42.4	55.0	37.7	42.6±2.9	
3	78, diseased	14	3	26.3 (0)	29.0 (0)	30.0 (1)	25.3 (1)				28.2±3.4	−0.4±4.8
	88, healthy			30.7	28.8	23.9	28.3				27.8±3.4	
4	98, diseased	14	3	25.8 (1)	23.1 (1)	31.6 (1)	10.7 (1)				26.0±3.4	6.6±4.8
	108, healthy			38.5	34.7	28.6	14.3				32.6±3.4	
5	600, diseased	13	4	25.4 (1)		13.2 (3)		3.8 (4)			17.0±4.0	5.3±5.7
	601, healthy			19.1		27.8		19.3			22.3±4.0	
6	612, diseased	15	4	28.8 (1)		31.0 (2)		18.5 (3)	15.8 (4)		25.5±3.5	6.2±5.0
	613, healthy			37.6		29.2		29.1	30.3		31.7±3.5	
7	614, diseased	14	4	9.4 (1)		15.3 (2)		23.5 (3)			16.0±3.7	8.8±5.2
	615, healthy			21.7		23.9		22.7			24.8±3.7	
8	620, diseased	15	4	33.7 (1)		35.1 (2)		28.0 (2)			32.5±4.2	5.1±5.9
	621, healthy			41.0		37.7		34.1			37.6±4.2	
9	622, diseased	13	4	0.3 (0)		27.0 (1)		21.5 (1)			16.3±5.4	11.7±7.4
	623, healthy			21.7		30.7		34.7			28.0±5.0	
10	634, diseased	15	8			−5.8 (2)		13.6 (3)	16.3 (3)		12.2±4.7	6.6±6.7
	635, healthy					25.2		20.4	14.4		18.8±4.7	
11	636, diseased	15	5	5.3 (1)		18.4 (2)		−15.3 (2)			4.9±4.4	13.2±6.3
	637, healthy			18.8		24.2		4.8			18.1±4.4	
12	644, diseased	18	11					26.7 (3)	7.4 (3)		15.2±5.9	6.4±8.4
	645, healthy							34.6	12.8		21.6±5.9	
13	646, diseased	13	8			20.4 (2)		17.4 (2)			18.0±5.9	12.4±8.4
	647, healthy					35.6		29.1			30.4±5.9	
14	1007, diseased	16	6			17.3 (2)		26.9 (3)	22.9 (3)	19.8 (3)	21.8±3.3	2.0±4.7
	2007, healthy					29.4		25.7	22.3	18.0	23.8±3.3	

[1] Description in text, p. 181. Numerals in parentheses indicate symptom intensity of attack by *C. lindemuthianum* as follows: 0=very slight lesions present on the veins of lower face of leaflet; 1=necrotic lesions plainly visible on veins of lower surface of leaflet; 2=necrotic lesions on most of veins of upper and lower surfaces of leaflet; 3=necrotic lesions present in lamina adjacent to infected veins and slight loss of chlorophyll around lesions; 4=advanced stage of 3, with pronounced loss of chlorophyll.

[2] Data taken between 8:90 a. m. and 12:30 p. m.

[3] Data taken between 12:30 p. m. and 5:00 p. m.

tion is found; this figure is highly significant. *Colletotrichum lindemuthianum*, therefore, reduced the assimilation of bean leaflets, as measured by the carbon dioxide utilized per unit area in unit time, by a significant amount.

No two inoculated leaflets showed the same response to fungus infection. In table 3, the consecutive stages in the development of symptoms on each leaflet are represented by the numerals 0, 1, 2, 3, and 4 placed in parentheses beside the assimilation reading. The presence of necrotic areas in the laminae of diseased leaflets did not reduce the assimilation rate as much as was expected from the symptoms observed. No measurements of necrosed areas per leaflet were made in the present investigation. A method is needed whereby daily measurement of diseased and healthy areas on a diseased leaf can be rapidly and accurately obtained without injury to the plant organ. Degrees of yellowing from loss of chlorophyll often characterized leaflets possessing necrosed areas. These symptoms may conceivably cause a reduction in the ability of cells to utilize carbon dioxide, but at present it is not known to what extent chlorophyll may be reduced in a cell before there is an apparent reduction in the rate of photosynthesis.

Respiration

Data on respiration were obtained from most of the leaflets listed in table 3, and are presented in table 4 as mean respiration rates per 5-day period. The mean of all readings per leaflet during the course of the observations is given in table 5. Each mean is the average of 2 to 4 individual 4-hour readings. The periods in table 4 include the same time intervals indicated in table 3.

Table 4.—*Mean respiration rates of pairs of opposite bean leaflets at four periods of maturity, when one member was healthy and the other was infected with Colletotrichum lindemuthianum, expressed as milligrams of carbon dioxide per 100 cm.² of leaflet surface per 4-hour determination*

[Each mean is the average of 2 to 4 individual observations]

Pair of leaflets No.	Leaflet No. and condition	Mean respiration rates of leaflets at indicated period of maturity[1]				Mean of all observations±S. E.	Difference±S. E.
		Period A	Period B	Period C	Period D		
		Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
1	14, diseased	7.2 (1)	9.7 (1)			8.7±3.0	3.1±4.3
	24, healthy	16.4	8.4			11.8±3.0	
2	36, diseased	14.6 (1)	13.0 (2)			13.8±3.3	5.1±4.6
	46, healthy	8.1	9.3			8.7±3.3	
3	78, diseased	9.7 (0)	19.4 (1)			13.6±3.6	1.0±5.1
	88, healthy	9.4	17.5			12.6±3.6	
4	98, diseased	15.3 (1)	8.4 (1)			11.9±3.3	2.3±4.6
	108, healthy	14.5	13.8			14.2±3.3	
5	600, diseased	9.7 (1)	4.1 (3)	1.6 (4)		6.4±2.5	2.7±3.6
	601, healthy	5.1	2.7	1.9		3.7±2.5	
6	612, diseased	9.1 (1)	11.8 (2)	4.6 (3)	0.2 (4)	7.0±2.4	8.4±3.4
	613, healthy	15.2	26.0	12.4	4.1	15.4±2.4	
7	614, diseased	18.1 (1)	23.4 (2)	10.7 (3)		18.0±2.5	12.5±3.6
	615, healthy	1.4	4.9	8.7		5.5±2.5	
8	620, diseased	19.8 (1)	6.1 (2)	22.5 (2)		12.4±3.0	7.0±4.3
	621, healthy	9.7	2.3	9.5		5.4±3.0	
9	622, diseased	12.8 (0)	12.8 (1)	34.5 (1)		17.2±3.5	4.8±5.0
	623, healthy	13.4	15.9	51.9		22.0±3.5	
10	634, diseased			7.9 (3)	12.7 (3)	11.5±4.0	8.8±5.7
	635, healthy			2.0	2.9	2.7±4.0	
11	636, diseased	12.3 (1)	7.0 (2)	5.3 (2)		8.4±3.6	2.1±5.1
	637, healthy	6.7	33.0	3.1		10.5±3.6	
12	644, diseased			13.2 (3)	15.7 (3)	14.4±4.0	1 2±5.7
	645, healthy			15.1	11.4	13.2±4.0	
13	646, diseased		7.8 (2)	8.7 (2)	20.6 (2)	13.3±3.6	2.4±5.0
	647, healthy		18.6	11.8	18.3	15.7±3.6	

[1] Description in text, p. 181. See also footnote 1, table 3.

Two pairs of opposite leaflets, namely, 612 and 613, and 614 and 615, showed significant differences between the respiration of the healthy member and that of the diseased member. The respiration of leaflet 612 (diseased) was less than that of leaflet 613, while the respiration of 614 (diseased) was greater than that of 615. There was no significant difference in respiration between healthy and diseased leaflets for any of the remaining 11 pairs of opposite leaflets.

Relative respiration rates of healthy and diseased bean leaflets at different stages of maturity are summarized in table 5. Diseased leaflets respired approximately 10 percent more carbon dioxide than healthy leaflets, but this difference is not statistically significant. For the plant material studied, it is concluded that infection with *Colletotrichum lindemuthianum* did not materially affect respiration. This somewhat unexpected result may be explained by assuming that necrosed areas respire less, and nonnecrosed areas, such as the margins of diseased spots, respire more than healthy tissues, and under the existing conditions these two opposite phenomena counterbalanced each other.

TABLE 5.—*Mean respiration rates of all healthy and all diseased leaflets for which data are shown in table 4*

Maturity of leaflets	Mean (±S. E.) respiration rate of—		
	Diseased leaflets	Healthy leaflets	Difference
	Milligrams	*Milligrams*	*Milligrams*
Period A	12.4±1.5	9.7±1.5	−2.7±2.1
Period B	11.6±1.5	11.2±1.5	−.4±2.1
Period C	9.9±2.0	11.3±1.9	1.4±2.8
Period D	12.3±2.7	8.5±2.7	−3.8±3.8
Average	11.6± .8	10.4± .8	−1.2±1.2

PHYSIOLOGY OF PAIRS OF DISEASED LEAFLETS

The disease selected for study on pairs of diseased leaflets was mildew, caused by *Erysiphe polygoni* DC., which appeared as tiny spots on the upper surfaces of a number of full-grown bean leaflets on June 10, 1934. It is not known when inoculation occurred or how many spores produced infection per leaflet.

APPARENT PHOTOSYNTHESIS

Two observations per day were obtained on apparent photosynthesis. These data, together with the number of mildewed spots per leaflet and the color changes of each leaflet due to the action of the pathogen, are given in table 6.

The presence of mildewed areas on a bean leaflet did not materially reduce assimilation unless yellowing was also present; excessive yellowing was accompanied by a pronounced reduction in assimilation as compared with the assimilation of healthy leaflets (table 1). Abscission of leaflets outwardly little affected by *Erysiphe polygoni*, was not uncommon; this phase of the disease is probably due to toxic substances produced by the fungus and not to withdrawal of food or water loss. It is of interest to note that the assimilation of leaflet 12 closely paralleled that of the companion leaflet 22, though the latter showed twice

TABLE 6.—*Daily assimilation and respiration rates of pairs of opposite bean leaflets infected with Erysiphe polygoni, expressed as milligrams of carbon dioxide per 100 cm.² of leaflet surface per 4-hour determination*

[Each figure denoting assimilation is the mean of two determinations]

Pair of leaflets No.	Leaflet No.	Mildew lesions	Assimilation and respiration rates of leaves on—												Color and condition of leaflets
			June 24		June 25		June 26		June 27		June 28	July 3	July 4	July 9	
			Assimilation	Respiration	Assimilation	Respiration	Assimilation	Respiration	Assimilation	Respiration	Assimilation	Assimilation	Assimilation	Assimilation	
		Number	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	
1	12	21	38.6	3.5	37.4	6.1	37.1	16.9	30.1	7.7	21.8	39.4	37.8	31.0	Dark green from June 24 to July 3, thereafter pale green.
	22	42	41.8	6.4	42.6	.8	48.8	5.6	35.5	12.5	29.7	32.2	33.5	21.6	Dark green from June 24 to June 28; thereafter pale green.
2	13	4			12.2		20.8	9.2	8.6	10.6	11.1				Pale green June 25; pronounced yellowing thereafter.
	23	6			27.2		20.3	9.2	25.5	2.4	8.8				Slight yellowing June 25; pronounced yellowing thereafter.
3	34	>7					4.8	8.6	19.7	12.0					Dark green June 26; pale green June 27; abscission June 28.
	44	>7					10.0	7.0	30.6	23.0	6.8				Yellowed.
4	95	>5	4.5	12.0	31.7	4.0	25.2	4.7							Pale green.
	105	>5	5.6	16.8	4.6	.5	11.4	6.6							Pronounced yellowing.
5	96	>5							7.2	21.3					Pale green June 27; abscission June 28.
	106	>7							−1.5	11.0	−2.4				Yellowed.
6	97	8			48.2	15.1	43.5		51.9						Dark green.
	107	10			29.6	10.6	32.2		37.6						Do.

as many fungous lesions as leaflet 12. The assimilation of these two leaflets per unit area resembles that of healthy leaflets. Outward symptoms did not always give an accurate indication of the effect of the fungus on assimilation; for example, leaflet 44 was considered to be the more diseased of the pair 34 and 44, but from the assimilation data leaflet 34 apparently suffered the greater internal damage.

Respiration

Data on the respiration of the mildew-affected leaflets are shown in table 6. The average respiration per 4-hour determination per leaflet is 10.6 ± 1.5 mg. of carbon dioxide per 100 cm.^{2} Healthy leaflets respired $10.4 \pm .8$ mg. of carbon dioxide per 100 cm.^{2} in a similar length of time (table 5). While the latter figure is not strictly comparable with the present data, the two results are so similar as to suggest that in the present experiments *Erysiphe polygoni* had little influence on respiration.

Apparent photosynthesis of bean leaflets injured by feeding of thrips

Measurements of assimilation were made on mature bean leaflets showing symptoms associated with the feeding of *Heliothrips haemorrhoidalis* (Bouché), the common greenhouse thrips. All stages of injury were present on the leaflets studied, from initial surface rasping to loss of chlorophyll in varying degrees. Plants bearing affected leaflets were fumigated with nicotine before determinations were started. Symptoms continued to develop after the thrips had been removed; the loss of chlorophyll increased until definite yellowing could be detected, and occasionally abscission of leaflets occurred. Abscised leaflets were generally dark green and outwardly fully turgid when leaflet fall occurred. This latent action of the feeding of the thrips is thought to be due to some toxic substance introduced by the insect. The fumigation with nicotine is not judged to have exerted any detrimental effect; healthy leaflets on the same plants as the thrips-injured leaflets showed no loss of chlorophyll or premature abscission. No data were obtained on the number of thrips that had fed on the leaflets or the length of the feeding prior to the start of physiological readings.

Photosynthesis readings for five pairs of leaflets, taken after feeding by thrips, are given in table 7; notes on the intensity of insect injury are included.

In figure 2, data obtained from an additional pair of leaflets (32 and 42) are presented. This pair of leaflets in a healthy condition was studied from May 23 to May 29, 1934. The rate of assimilation of the two leaflets was very similar and the difference between their mean assimilation rates is not significant (table 1). The plant bearing the leaflets was transferred to the greenhouse on May 30, and on June 12 both leaflets showed evidences of the feeding of thrips. The plant was fumigated with nicotine and returned to the assimilation chamber, where further determinations were made at intervals until June 23.

The data presented in table 7 and in figure 2 show that an appreciable reduction (43 to 80 percent) in the assimilation rate followed the feeding by thrips. However, this lowering of the assimilation rate undoubtedly includes reductions due to natural senility, for these leaflets were older than any of the other leaflets studied.

Reductions in assimilation were proportional to the apparent severity of the insect injury.

TABLE 7.—*Daily assimilation rates of pairs of opposite bean leaflets that had been injured by the feeding of Heliothrips haemorrhoidalis, expressed as milligrams of carbon dioxide per 100 cm.² of leaflet surface per 4-hour determination, with notes on the severity of insect injury, 1934*

Pair of leaflets No.	Leaflet No.	Type of injury [1] and daily assimilation rate of leaflets on—							
		May 19		May 20		May 21		May 22	
		Type of injury	Assimilation rate	Type of injury	Assimilation rate	Type of injury	Assimilation rate	Type of injury	Assimilation rate
			Milligrams		*Milligrams*		*Milligrams*		*Milligrams*
1	31	R	45.2	R	41.7	R	39.5	R	35.1
	41	R	39.1	R	54.1	R	41.5	R	67.8
2	72			R	55.1	R	33.8	RR	22.9
	82			R	21.8	R	28.0	R	29.8
3	76	R	28.2	R	17.9	RR	23.3	RR	22.5
	86	R	16.6	R	33.6	R	21.6	RR	3.1
4	91	RR	9.2	RR	21.6				
	101	R	20.9	R	45.4				
5	92							RRR	24.5
	102							RRR	9.4

[1] R=rasping of leaflet surface with little or no loss of chlorophyll; RR=rasping accompanied by slight yellowing; RRR=rasping accompanied by much yellowing.

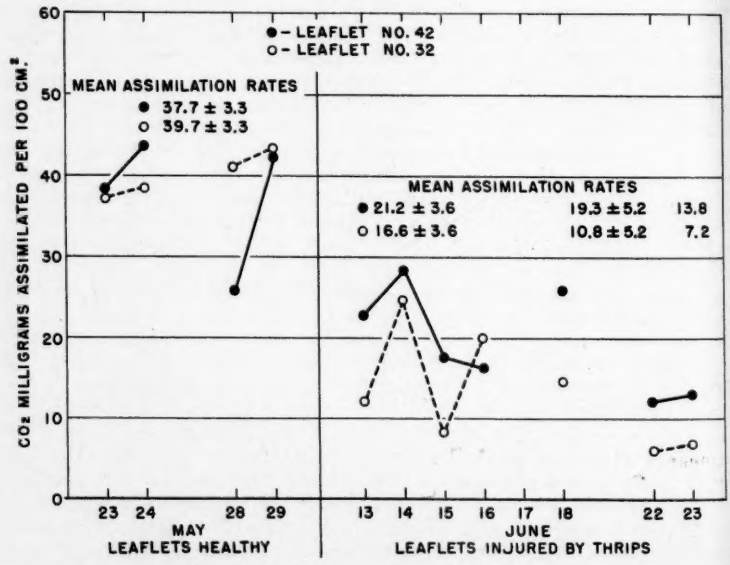

FIGURE 2.—Assimilation per 4-hour period of a pair of opposite bean leaflets (Nos. 32 and 42), both healthy, May 23–29, 1934; both injured by thrips June 13–23. Expressions of insect injury were as follows: Rasping with little loss of chlorophyll: Leaflet No. 32, June 13–15; leaflet No. 42, June 13–17. Slight yellowing: Leaflet No. 32, June 16–18; leaflet No. 42, June 18–21. Pronounced yellowing: Leaflet No. 32, June 19–23; leaflet No. 42, June 22–23.

SUMMARY

Between the members of a pair of healthy and opposite bean leaflets, there are no statistically significant differences in (1) assimilation of carbon dioxide per unit area in unit time, or (2) growth, as measured by either increase in area or total area. Between different pairs of healthy leaflets on the same or on different plants, significant differences in assimilation are occasionally found. Differences greater than 15 percent are considered statistically significant.

The assimilation rate of healthy bean leaflets, even on a unit-area basis, increases with maturation of the leaflets. Studied for 15 consecutive days, with determinations started 13 to 15 days after seeds were planted, leaflets assimilate progressively greater and greater amounts of carbon dioxide. The maximum point in the assimilation curve in relation to age of the bean leaflet was not determined.

Leaflets infected with *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav., assimilate 24 percent less carbon dioxide than healthy companion leaflets, and are restricted in growth by approximately 1.6 percent. Both of these differences are statistically significant. No statistically significant difference has been found between the respiration of diseased and healthy leaflets.

Erysiphe polygoni DC. does not reduce normal assimilation until yellowing, due to the action of the fungus, is produced. Diseased leaflets often abscise prematurely while still dark green and turgid. No significant difference has been found between the respiration of mildew-affected and healthy leaflets.

Feeding injuries by thrips (*Heliothrips haemorrhoidalis* (Bouché)) cause a loss of chlorophyll and yellowing and an appreciable reduction in the assimilation rate; the reduction is proportional to the apparent severity of the insect damage.

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